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Cell therapy manufacturing: process analytic technologies needed to achieve flexible, feedback-driven automation

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Abstract

Cell therapy continues to change the therapeutic landscape in multiple fields, from cancer to monogenetic diseases. One key pain-point for increasing access to cell therapies has been developing manufacturing processes for different products. In addition, the growth in commercially viable cell therapeutic products has been hampered because of a lack of manufacturing platforms flexible enough to accommodate multiple therapeutic processes. New automated manufacturing platforms are emerging to address the current deficiencies in the cell therapy manufacturing market, but further development is required to integrate additional analytical technologies. Expanding a given manufacturing platform's capabilities is likely to involve the addition of process analytical technologies to enable feedback–driven processes and change the method by which manufacturing processes are locked. Process analytical technique integration will help lower therapeutic costs, improve product quality, and limit the failed batch rate thereby enabling increased patient access to curative therapies.

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Introduction

Cell therapies continue to reshape oncology and other therapeutic areas. However, as this shift in treatment modalities is accelerating, challenges are becoming apparent. One such area of challenge is manufacturing. New closed-system processing manufacturing platforms are coming to market focused on addressing specific cell therapy manufacturing pain-points. A subset of cell therapy manufacturing platforms automates multiple process steps serving to reduce therapeutic costs, enable efficient scaling, and maintain product quality. Pain-points remain regarding what technology combinations are needed to design adaptable manufacturing platforms to meet the manufacturing needs of various therapeutic modalities (i.e. T-cells, Natural Killer cells, Mesenchymal Stem Cells, inducible Pluripotent Stem Cells, and so on). As new technologies emerge, multiple technologies can be linked together for a modular approach. Alternatively, this vision can be realized where one platform (or 'box') can integrate many technologies and adapt to multiple manufacturing modalities. Some advantages of a fully integrated single system are the standardization of the set-up, ease of use, and ease of data analysis, especially if the information produced requires complex algorithms to dictate the feedback mechanism. Deeper sensors and other PAT integration will be required for current and future manufacturing platforms to realize their full potential. Pursuing additional PAT integration will result in a cellular activity feedback-driven platform, thereby enabling processes to be modified in real-time. Most current manufacturing processes are locked based on a pre-defined schedule. For those that are using analytics to dictate process duration, off-line measurements are required, increasing labor and the chance of contamination. In complex biologics manufacturing (i.e. autologous CAR-T), where patient starting material can vary significantly, additional PAT integration will enable more robust, adaptable processes.

In addition, this will allow a tighter level of control over complex cell therapy manufacturing processes and allow the early detection of poor process performance [1]. Manufacturing runs will also yield larger, more detailed culture datasets, which can ultimately be analyzed for

further insight. This will result in more curative therapies being offered to patients at a lower price.

Flexible cell therapy manufacturing

Cell therapy manufacturing has significantly evolved, recently, moving from predominantly open and manual manufacturing solutions to multiple closed-cell processing platforms. New closed, automated, and scalable manufacturing solutions have come to market, addressing several pain-points. While this shift in the market is good progress, additional PAT integration is needed to increase manufacturing platform adaptability and further close the process.

Rationale to migrating toward feedback-driven technologies

The two types of cell therapy manufacturing are autologous and allogeneic. The current closed, automated, and scalable cell therapy manufacturing platforms have limited ability to offer real-time culture data beyond pH and dissolved oxygen (DO) or implement feedback-driven automation [2]. While these are useful data points, they do not provide in-depth information on phenotype, functionality and other important characteristics. Allogeneic cell therapy manufacturing, involving the production of large cell numbers capable of producing doses for multiple patients, can use a broader range of sensors and PATs. At this scale, it enables more expensive, potentially reusable PAT technologies, to be used where their cost is amortized over a large dose number [1,2]. The considerations about whether allogeneic process PATs are disposable hinge on contamination factors and how multiple cleaning cycles might affect the technology. Furthermore, allogeneic therapies use well-characterized cells and so the manufacturing process has been comprehensively optimized, leading to less variability. Conversely, autologous cell therapies offer a unique challenge. In autologous cell therapies, a manufacturing process yields a therapeutic product for a single patient. Because of this, any integrated PATs are preferably disposable and cheap to maintain low therapeutic costs. Manufacturing autologous cell therapies is more difficult because of the variability observed between patients' starting material, which makes process monitoring and flexibility increasingly more important. Unlike allogeneic processes, which start with a prescreened cell source, autologous treatments start with cells from patients suffering from diseases. As the cell performance can vary based on the health of the patients, there is greater variability between batches, increasing the need for technologies that offer feedback to control the process and generate a similar outcome.

Reusable sensor and process analytical technology

Reusable sensors and PATs bring the highest value in large batch manufacturing processes, such as allogeneic cell therapy manufacturing because their cost is spread over a large number of doses. These probes are intended to be reused, causing them to be more expensive, but enable various culture condition measurements using a single probe, including pH, DO, cell number, and other metabolites [3]. One such sensing technology is Raman spectroscopy where a probe is aseptically inserted into the bioreactor to measure various growth environment conditions [1]. Raman's primary advantage is its sensitivity and ability to enable the measurement of multiple process attributes simultaneously. There are drawbacks to the Raman technology, including an upfront effort to develop and optimize an algorithm for each process [1]. In addition, incremental algorithm optimization is required for each additional process or when a change is made to an existing process. While there is increased upfront effort, once the algorithm is optimized for a particular process, minimal additional effort is required. Because of the technology's sensitivity and complexity, Raman systems and probes are expensive, reducing their usefulness outside large batch processes. The combination of cost and ongoing algorithm optimization make reusable PATs, such as Raman unsuitable for autologous cell therapy manufacturing, requiring another option.

Single-use sensors and PATs

The availability and selection of single-use PATs, including sensors, are one pain-point in cell therapy manufacturing. Aside from pH and DO optical sensors enabling real-time measurements in subsets of manufacturing platforms, few other methods exist to assess growth environment conditions and/or metabolites on-line or in-line. The greatest need for single-use sensor and PAT technologies is in autologous cell therapy manufacturing. Globally, autologous cell therapy programs make up most of the cell therapies in development, all of which would benefit from additional manufacturing PATs [4]. Various companies are working to develop PATs to enable real-time cell counting, identity, and metabolite sensing. Although there is considerable work in this space, many PATs, particularly metabolite sensors, are falling short of expectations. Some sensors, including those measuring glucose and lactate, seek to monitor and maintain an optimal growth environment. Sensor drawbacks generally include questions about sensing lifespan and the dynamic range. Two broad sensor classes are being developed, one class is optical sensors, much like many existing pHs and DO sensors, while others are chemical reaction based. Optical metabolite sensors would be preferred as they have a long sensing lifespan assuming the sensor is light-shielded. Chemical-based metabolite sensors are less desirable because their sensing lifespan is contingent on sensor substrate availability, potentially limiting their use in longer manufacturing processes that require more than several weeks of culture duration (i.e. induced pluripotent stem cells). Separate but equally important is the sensor dynamic range which, if too narrow, limits their usefulness in detecting real-time metabolite changes. Glucose sensors should be capable of accurately

measuring between 0 and 30 mM, the concentration of high glucose media. Lactate sensors should also be capable of accurately measuring within a broad, of 0–30 mM, observed during cell culture. Many of the other factors present in culture media (e.g. glutamine, ammonia) are present at lower concentrations are challenging to accurately measure using single-use technologies. A further pain-point within the disposal PAT space is the lack of sensing options that enable multiple metabolite measurements embodied in a single sensor or probe.

A search is underway in autologous cell therapy manufacturing for PATs which can readily adapt to various processes. The reason for this is fairly simple, there is no 'standard' autologous cell therapy process, with most processes having different requirements and reagents. Additional PATs are necessary for autologous cell therapy manufacturing to increase platform adaptability to not only differing processes but also patient starting material variability. Implementing additional PATs opens the possibility of changing the way manufacturing processes are locked, moving away from schedule-locked processes to ones based on cellular activity.

Feedback-driven cell therapy manufacturing

As cell therapy manufacturing technologies continue to mature, PATs are needed to enable feedback-driven manufacturing processes. For example, DO and pH sensors are currently being used to control processes in real-time. As cells proliferate, the pH can decrease. To correct this, the carbon dioxide concentration in the environment can automatically decrease to increase the pH levels. Alternatively, low pH values can trigger a media exchange. Similarly, with expanding cells, DO levels will decrease and the gas exchange in the system can be increased to compensate for this. Integrating these feedback mechanisms helps to maintain healthy cells in an environment with the correct physiological targets. DO and pH control is currently in use in automated closed–cell therapy manufacturing platforms. However, additional analytics are required to better control processes. Currently, these can be performed by using off-line technologies, such as flow cytometers and enzyme-linked immunosorbent assays. These are open manual processes, primarily designed for higher throughput research and development rather than closed system manufacturing. Using these types of equipment can result in an increased risk of errors and an increased risk of contamination or process disruption. Many of the current technologies required highly skilled employees to operate and analyze the data resulting in higher costs. To obtain a broad range of analytical results, several pieces of equipment are required, which requires additional space in the cell therapy manufacturing facility. This further increases the costs. To fully realize a vision of reducing manufacturing/therapeutic costs, improving quality, and decreasing batch failures, feedback-driven process automation will be critical. PAT integration into these manufacturing platforms will require at-line and/or in-line integration to enable efficient scaling.

PAT integration with closed, automated manufacturing platforms

Closed, automated cell therapy manufacturing platforms will benefit from additional PATs including sensing technologies, to enable further touch-point reductions and more stable, adaptive processes [5,6]. Furthermore, PAT integration will produce 'smart' manufacturing platforms able to make decisions based on pre-defined process parameters to maintain an optimal growth environment. Enabling smart decisions would improve process adaptability and the ability to perform automated media exchanges based on growth environment metabolite concentrations. This would shift how processes are locked for manufacturing from a predominantly pre-defined schedule to one predicated on cellular activity. This represents an important step in cell therapy manufacturing, particularly in autologous cell therapy manufacturing where significant variability in patient starting material exists. In the current absence of these additional integrated PATs, it is challenging to develop a robust, closed, adaptive autologous cell therapy manufacturing process. In particular, it is difficult to anticipate how patient starting material variability might influence a final product's critical quality attributes (CQAs) leading to increased batch failures. A method to mitigate this is shifting to processes locked based on cellular activity rather than a schedule. Furthermore, enabling automated, easy-to-use and integrated PATs would support the production of a larger amount of data, enabling the correlation between in-depth cell characteristics and patient outcomes. Understanding how the cell characteristics relate to patient outcome would provide data to further refine CQAs, which can then be used to implement process improvements through enhanced feedback. Improving the specificity of the target CQAs could potentially lead to improved patient outcomes through improved feedback mechanisms. Further work is required to identify the optimal CQAs and the corresponding processing parameters to achieve these targets.

Additional process analytical technology manufacturing integration

Metabolite sensor integration into closed, automated, scalable manufacturing platforms is critical to make them 'smarter' but there are other PATs that can improve automation, process robustness, and adaptability. One technology frequently discussed is in-process, real-time cell counting to provide a non-invasive method to assess total or viable cell numbers. Various technologies can be leveraged to measure cell numbers in real-time, including imaging, fluorescence,

flow cells, microfluidic chips, impedance, spectrophotometry, metabolite measurements and even Raman spectroscopy [7]. More than one of these options require resuspending cells in culture to gain an accurate count. Cell counting technology which obtains data without disturbing cells in culture provides the least invasive option, potentially resulting in improved cell performance. Indirect correlations of metabolite production or consumption offer a non-invasive method of cell number. However, these methods require further advancement as shifting cell metabolism, from aerobic to glycolytic, can require more complex algorithms with multiple targets measured simultaneously to accurately predict cell numbers. High-value PATs for integration into the manufacturing platform includes cell identity, protein expression, and functionality technology. Depending on the level of integration, this may be the most complex technology to integrate. In one scenario, a manufacturing platform might leverage an off-line automated flow cytometer for cell identity to significantly cut hands-on time and turnaround time for samples, such as Accellix [8]. This would require the automated cytometer to be reagent flexible as each cell therapy process is unique regarding the cell surface targets assessed. Something else to consider is aseptically connecting manufacturing platforms at-line to flow cytometers for cell transfer and data collection [9]. As with the prior configuration, this setup would require manual intervention, limiting potential cost savings but provides a modular, flexible solution that is adaptable to different processes. The most complex solution is full in-line cell identity integration. While complex, this would provide the biggest potential costs savings and represent an all-in-one solution but comes with significant risks in manufacturing platform cost and complexity.

Quality control and product release testing

Streamlined PATs are not only needed during manufacturing but also in other cell therapy logistics, including quality control (QC) testing and product release. In an effort to decrease both costs and vein-to-vein times, cell therapy manufacturing processes are becoming shorter. Currently, if a therapeutic developer has a short process and some are approaching 24 or fewer hours in length, QC release testing is still required before the final product can be released to the patient. This typically adds 7–10 days, meaning the final product must be frozen and stored while QC testing is completed, delaying patient dosing [5]. Further PAT development is required to streamline QC release testing if vein-to-vein times are to be appreciably shortened.

Manufacturing methods — schedule lock versus cellular activity lock

As processes are further automated and streamlined with additional PAT integration, a discussion will begin on whether current manufacturing methods are optimal. In allogeneic cell therapy manufacturing, healthy donor starting material or master cell banks generally have low variability. Autologous cell therapies rely on the patient's starting material and do observe considerable variability. This variability originates from both their specific cancer indication and lingering effects from prior lines of therapy, challenging the ability to manufacture products consistently. Further complicating matters, autologous cell therapy manufacturing processes are developed primarily using healthy donor material, which is not the optimal surrogate for patient starting material. When cell therapy manufacturing processes are locked for clinical manufacturing, they are locked on a pre-defined schedule basis. As additional PATs are integrated into autologous manufacturing platforms, a discussion is needed on whether processes are locked based on a predefined schedule. Because of the starting material variability, autologous manufacturing processes would ideally be locked based on cellular activity. Basing process decisions on cellular activity may serve to reduce batch failures as some patients' cells need additional time during expansion to meet release criteria while others may meet release endpoints early, reducing vein-to-vein time. In moving to processes locked based on cellular activity, the goal of achieving reduced costs, lowering batch failures, and increasing product quality comes closer to reality.

Conclusions

While cell therapies are reshaping the treatment paradigm in oncology and other disease areas, manufacturing continues to be a pain-point. For the field to continue its evolution, manufacturing solutions must evolve as well. Part of this evolution will be deeper PAT integration into manufacturing platforms. Allogeneic cell therapy manufacturing processes primarily implement complex, reusable PAT solutions due to using low-variability healthy donor starting material and large batch size.

Therefore, the primary need for additional PAT integration lies with autologous manufacturing platforms. Because autologous manufacturing processes result in a single therapeutic product for each patient, any PAT solution must be cheap and disposable. This is a current challenge developing solutions with sufficient sensitivity and dynamic range while being low cost and disposable. Even so, there are options beginning to emerge which offer additional real-time, in-process sensing capabilities. The integration of additional PAT solutions into new and existing closed, automated manufacturing platforms will be vital in lowering therapeutic costs while maintaining product quality and reducing batch failures. As these PATs are integrated into various platforms enabling 'smart' manufacturing decision-making, a discussion is needed on whether we

are locking processes correctly. Because most of the uncertainty in autologous manufacturing originates from the patient starting material, locking processes based on a pre-defined schedule may not make sense. The integration of additional PAT solutions into manufacturing platforms will enable processes to be locked based on cellular activity. This should be the ultimate goal as it will reduce manufacturing costs, maintain product quality, and lead to reduced batch failures. Ultimately the goal of developing feedback-driven automation platforms for manufacturing should be providing increased patient access to curative therapies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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