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Are automated disposable small-scale reactors set to dominate the future of pharmaceutical bioprocess development?

It is predicted that automated small-scale disposable reactors will dominate bioprocess development over the next ten years and beyond.

Keywords: disposable bioreactors, end-to-end bioprocessing, high-throughput process development

Biologics and vaccines continue to gain momentum with over 400 monoclonal antibodies in development and the expansion of new modalities, such as antibody-drug conjugates, glycanengineered proteins, multispecific targets and novel fragments [1]. This comes at a time when the biopharmaceutical industry continues to face tough pressures including cost control of not only manufacturing but also drug development. It is well established that high-throughput methodologies can provide efficiency improvements to speed drugs to market and minimize development resources [2]. Such improvements have been achieved in cell line development by the integration of single-use disposable technology with automation [3]. Examples include the use of disposable deep-well plates for clone screening supported by automated liquid handling [4], and the use of spin tubes, with vented caps at 50 ml scale, as suitable mimics of shake flask performance [5]. The spin tube operation has led to automated cell passaging workflows using liquid handlers with tube capping/decapping, pH adjustment and cell viability counting [3]. This eliminates the need for the laborious and mundane shake flask passaging. Final clone selection and media screening can now be completed in a high throughput manner using arrays of 24 disposable reactors (15 ml) with liquid handling capability [6]. These successful efficiency gains for cell line development have focused attention to improving the speed of upstream process development by the integration of disposable reactors with automation. These automated upstream tools form part of an end-to-end approach for high-throughput development of a complete process. The workflow includes liquid handler based purification such as phytip or miniature columns containing capture resin [7]. These are coupled with high throughput analytical methods for rapid glycan's, UPSEC [8], and rapid microfluidic ELISA [9].

It is well established that the development of biologics or vaccines requires a multifactorial statistical experimentation (DOE) approach. Typically two level factorials are used to understand the complex interactions where optimum conditions for titer often compete with those desired for quality attributes [10]. For upstream development this requires iterative rounds of labor intensive statistical experimentation of at least three–six parameters requiring approximately 20 reactors per study [11]. There is an efficiency drive for process development to be 'right first time' and avoid unnecessary repeats of work in late-stage development. In addition, the focus on QbD initiatives and characterization enables understanding of the process design space and failure limits. Traditionally, this resource burden has been carried out at the lab scale with 3 l glass to 30 l stainless steel reactors. The operating ranges of the laboratory



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reactors, such as power per unit volume and gas flow rate regime, are deliberately limited to match that of commercial-scale reactors so that a true scale down mimic is achieved. The long-duration process (10–21 days) also requires sufficient broth volume for a comprehensive product quality analysis including glycoforms, potency, aggregation and purity. Transitioning this bioreactor work to a single-use disposable format enables the benefits of fast turnaround between experiments by reducing reactor clean up, setup, and eliminating steam sterilization cycles.

To allow continuity for line of sight to commercial manufacturing, it is essential that such small-scale disposable reactor designs are aligned with the standard stirred tank geometry used throughout the industry and avoid novel designs that are only applicable to laboratory scale. The small-scale reactor technology must enable process development with the confidence that the scale down model mimics commercial manufacturing. Over the last 5 years a number of examples have shown the feasibility of designing miniature stirred tank reactors that could mimic the large-scale performance using the standard bioprocess engineering correlations such as power/volume [12,13]. These efforts provided inspiration that the vision of high-throughput automated upstream process development could be achieved by combining the benefits of small-scale single-use disposable stirred tank reactors with liquid handling automation. Recently, Merck acted on this potential and collaborated with TAP BioSystems to design an innovative solution of the small-scale disposable reactor system (250 ml) arrayed with 24 automated reactors [14]. This dual-use system for either cell culture or microbial processes was a considerable technical accomplishment given the diversity of growth rates and parameter control needs of the high cell density processes. The system enables a single scientist to run a DOE study with 4-6 parameters simultaneously across 24 reactors for a typical CHO mAb duration of 10-14 days. This compares to the traditional approach of a set of six glass reactors assigned to one project, taking 6-8 weeks to cover the 20-24 runs of the DOE. The automated reactor system has a user friendly graphical interface that enables fully automated independent operation of each reactor. This includes sophisticated feeding regimes and event triggered activities such as feed addition after reduction of oxygen uptake rate. The automated sampling from the 24 reactors generates a large sample analysis burden especially as profiling of key components such as glucose, lactate, osmolality, dCO, and cell viability is essential to upstream development. Therefore, advances are being made to integrate reactor technologies with the necessary metabolite and cell bioanalzyers. Over the next few years, it is anticipated that further efficiency improvements will be made with the integration of single-use sensors such as near IR, solid state pH and immobilized sensors into disposable and stainless reactors [15]. In addition, new approaches to more efficient experiment design are likely to be adopted, such as improved algorithm tools that allow smaller data sets to identify optimal conditions than with current factorial approaches [16].

The efficiency gains of automated high-throughput process development can enable a faster throughput of pipeline projects and also allow larger experiment designs to improve process understanding. Currently, the experimental burden using traditional 3 l glass reactors limits the number of possible studies. This often results in a partial understanding of the design space that barely touches the failure limits of a process. Expanding the DOE characterization studies over the failure limits should widen the process design space, and lead to enhanced reliability for commercial manufacturing. This is especially important for new modalities such as antibody–drug conjugates and bispecifics, where the end user must evaluate possible deviations from existing platform processes. As the adoption of the automated disposable systems expands it is anticipated that applications will widen to include operations such as microcarrier processing, perfusion and protein folding.

The adoption of single-use technology requires the end users to rely heavily on the ability of the suppliers to provide reproducible quality of a γ -irradiated product without faults or supply interruption. This requires end users to partner with suppliers to build trusting technical and business relationships. The supply chain security is vitally important to avoid delays to aggressive project timelines. Suppliers recognize these concerns and are derisking by using two sources for key components such as resins and tubing manifolds. Encouraging

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progress is being made across the industry to harmonize approaches for supply chain, standardization and connectivity. For example, a number of industry organizations such as the Disposable Working group of the BioPhorum Operations Group (Sheffield, UK) [101] and the BioProcess Systems Alliance (Washington DC, USA) [102], are working to provide harmonized extractable/leachable testing, improve the supply chain and define paths towards equipment standardization. In addition, a number of companies including Merck have their own cross-divisional communities of subject matter experts to support coordinated approaches for implementation and standardization of single-use technology.

In summary it is predicted that automated small-scale disposable reactors will dominate bioprocess development over the next ten years and beyond. This will provide a new era of efficiency gains in the range of three-six times faster over current approaches. This accelerated process development will continue to support our commitment for improving the speed of providing drugs to patients using reliable and robust processes. In the future it is possible that further efficiency gains may become feasible by applying microfluidic approaches to process development. However, the translation of single cell type systems to large-scale manufacturing remains a significant challenge.

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