Advances in High-Throughput Microscale Bioreactors (Postprint)

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Abstract

In recent years, mammalian cell culture technology has advanced rapidly, and the biopharmaceutical industry built upon this technology has risen prominently. In the fiercely competitive biopharmaceutical market, shortening research and development (R&D) time and reducing R&D costs are critical to success. Compared with conventional bioreactors, high-throughput micro-bioreactors offer advantages including operational simplicity, high throughput, and excellent experimental reproducibility, which can substantially shorten the R&D cycle and reduce labor and material costs; consequently, they have emerged as one of the latest research focuses in the biopharmaceutical industry. Currently, micro-bioreactors that have been successfully applied in biopharmaceutical R&D include SimcellTM, Ambr 15TM, and Ambr 250TM, each applicable to different stages of process development. This article will use these three micro-bioreactors as examples to introduce the current research status and future development prospects of high-throughput micro-bioreactors in mammalian cell culture process development.

Full Text

Preamble

High-throughput Micro Bioreactor Development for Biopharmaceuticals

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Abstract: The development of biologics based on mammalian cell culture technologies has seen increasingly rapid advances for the pharmaceutical markets in recent years. Economic concerns and time constraints as critical factors

and driving forces have accelerated bioprocess development for delivery of new biopharmaceutical drugs to market. Dramatically, advancement of semi-high-throughput micro-bioreactors in bioprocess development has shown a significant alternative for conventional approaches due to automation, increased capability of throughput, and excellent parallel level compared to costly and laborious bench-top bioreactors.

There are several commercially available micro-scale bioreactors, such as SimcellTM, Ambr 15TM and Ambr 250TM, being applied in different stages of cell culture development to enhance throughput. This research reviewed and summarized the strengths and challenges of high-throughput bioreactors for mammalian cell culture, showing the potential as scale-down models for process development and further improvement in the future.

Keywords: Mammalian cell, Cell culture process development, Bench-top bioreactor, Micro-bioreactor

1.1 Biologics Development Pipeline

In recent years, rapid advances in biotechnology have fueled vigorous growth in the biopharmaceutical industry. The expiration of adalimumab patents in 2015 —the first fully human monoclonal antibody—has reignited research into novel biologics and biosimilars, with a new peak expected by 2025 [1]. According to 2016 pharmaceutical sales data, eight of the top ten best-selling drugs worldwide were biologics [2]. Currently, over 400 biologic drugs are under development, including antibody-drug conjugates, bispecific antibodies, polysaccharide-engineered proteins, and novel target biologics [3], the vast majority of which are produced in mammalian cells.

Generally, biologics manufactured via mammalian cell culture undergo preclinical research and three phases of clinical studies (see Figure 1 [Figure 1: see original paper]). Preclinical research involves cell line construction, cell culture process development, purification process development, and formulation optimization. This phase offers considerable flexibility for implementing strategies to improve efficiency, reduce development time and costs, and accelerate market entry. Various high-throughput micro-bioreactors have emerged to facilitate rapid clone screening and culture process optimization. Meanwhile, different design philosophies for high-throughput micro-bioreactors balance operating volume with throughput capacity, accommodating diverse research and development strategies.

1.2 Advantages of High-throughput Micro-bioreactors

Traditional cell culture process development typically involves clone screening, process optimization, and scale-up. This work is usually performed in shake flasks and 1-15 L bioreactors, requiring 7-10 experimental rounds (one round representing one culture cycle) to select final clones from numerous candidates while optimizing multiple parameters such as pH, media, feeding strategies, temperature, and inoculation density. The entire process averages 9-12 months, is time-consuming, labor-intensive, and costly. Moreover, due to numerous experimental conditions, traditional bioreactors struggle to conduct multi-factor, multi-level experiments simultaneously, limiting their application in high-throughput rapid development.

To overcome these limitations, high-throughput micro-bioreactors have emerged [4]. These systems dramatically reduce culture volume and costs while increasing the number of reactors that can be operated per cycle (see Table 1), while retaining control over multiple critical parameters. Compared to shake flasks—the higher-throughput model used in conventional process development—micro-bioreactors incorporate culture environment control systems and feature geometric designs that more closely resemble conventional reactors, facilitating easier process scale-up.

Compared to traditional controlled models such as 3 L bioreactors, micro-bioreactors not only reduce material consumption and R&D costs but also offer high-throughput capabilities that can be effectively combined with Design of Experiment (DoE) approaches to shorten development cycles. Our platform' s experience demonstrates that early-stage process development using high-throughput micro-bioreactors requires only 3-6 months, reducing development time by 30-50%.

Although micro-bioreactors still differ somewhat from traditional reactors in structure and control, numerous studies have shown [5] that cell growth and metabolic performance are highly consistent between the two models, and processes developed in micro-bioreactors can be successfully reproduced at pilot and production scales. Thus, high-throughput micro-bioreactors can replace traditional reactors for labor-intensive work, representing an important future trend in cell culture process development.

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2 Models and Applications of High-throughput Micro-bioreactors

Numerous high-throughput micro-bioreactor systems exist. Early products such as microplates [6] and 24-deep-well plates [7-9] had microliter-scale volumes and were primarily used for initial clone screening, but their inability to monitor critical culture parameters limited their applicability and they have gradually been phased out. Currently, three widely used high-throughput micro-bioreactors

are SimcellTM, Ambr 15TM, and Ambr 250TM, each with different culture volumes suited for different stages of cell culture process development. Specific parameters are shown in Table 1.

Table 1. Parameters comparison between high-throughput mini-bioreactors and bench-top bioreactors [5,17,22]

Items	Bench-top Bioreactors (1~3L)	Shake Flasks	SimcellTM	Ambr 15TM	Ambr 250TM
Quantities	Less*	50-1000mL	7000m	10-15mL	200-250mL
Volume	Manual	Automated	Automated	Automated	Automated
Capital cost	Large footprint	Moderate	Large footprint	Low footprint	Low footprint
Temperature control	Individual	Incubator	Controlled in units of 252	Controlled in units of 12	Individual control
pH control	Real-time	Periodic	Real-time	Real-time	Real-time
DO control	Real-time	Periodic	Real-time	Real-time	Real-time
Gassing	Overlay + Sparger	Surface	Overlay + Sparger	Overlay + Sparger	Overlay + Sparger
Oxygen KLa	2-10h [1]	2.6~6.0h [1]	2.5-8.5h [1]	2-10h [1]	2-10h [1]
Agitation	200~300rpm	100~125rpm	300~1500rpm	200~800rpm	200~300rpm
P/V values	30-70W/m^3	3.9-419W/m^3	10-445W/m^3	40W/m^3	30-70W/m^3
Agitator blade	Three-blade propeller	Two-blade propeller	Three-blade propeller	Three-blade propeller	Three-blade propeller
Mixing time	10-100s	5-25s	5-40s	10-100s	10-100s

*Based on the maximum available quantities of a skilled scientist in one round of study

2.1 SimcellTM

Each SimCellTM bioreactor (BioProcessors Corp, USA) has a total volume of less than 800 L. As shown in Figure 3 [Figure 3: see original paper], the system comprises five culture plates, each independently controlling temperature, humidity, gas composition, and CO partial pressure (pCO). Aeration is achieved through a permeable membrane at the top of each reactor, with oxygen mass transfer coefficient (KLa) reaching 7 h^{-1} and CO KLa reaching 20 h^{-1}. The system enables online, non-invasive measurement of cell density (CD), pH, and dissolved oxygen (DO). Each culture plate holds six culture modules, with each module simultaneously running 42 micro-bioreactors, theoretically allowing up to 1,260 micro-bioreactors to operate simultaneously on a single SimcellTM system.

Leveraging its ultra-high throughput capability, SimcellTM can screen hundreds of culture conditions in a single experimental round. Rachel et al. [10] used a full factorial design to evaluate multiple factors including feed ratio, pH, DO, and glutamine supplementation in a single round of experiments, completing process optimization for CHO-K1SV cell culture in just one run. SimCellTM also demonstrates excellent parallelism among micro-bioreactors within the same module. Ashraf Amanullah et al. found that the coefficient of variation for culture parameters across different modules and individual micro-bioreactors was within 10%, indicating good reproducibility [11]. Additionally, SimcellTM has proven to be a scalable model. Rachel Legmann et al. achieved consistent results using SimcellTM at scales 2,000-3,000 times larger [10].

2.2 Ambr 15TM

Ambr 15TM (Sartorius Stedim Biotech, UK) is a single-use micro-bioreactor with a rectangular geometry and baffled mechanical design featuring eccentric agitation. This design simplifies structure, reduces manufacturing costs, and provides mixing characteristics similar to traditional baffled cylindrical bioreactors. Independent gas lines and optical sensor patches for pH and DO enable effective control of agitation speed, temperature, pH, and DO during culture. With a culture volume of 10-15 mL, it can sufficiently support subsequent productivity and quality parameter analysis. An Ambr 15TM system includes two or four workstations, as shown in Figure 4 [Figure 4: see original paper], with each workstation automatically controlling 12 micro-bioreactors simultaneously. Except for temperature and agitation speed, which are controlled uniformly across all 12 reactors, each reactor can independently set other process parameters, providing control capabilities essentially equivalent to traditional 1-3 L bioreactors. Furthermore, culture operations such as sampling and feeding can be automatically implemented through programmed robotic arms.

Recent studies demonstrate that Ambr 15TM not only increases throughput and reduces costs but also shows good consistency with traditional process develop-

ment reactors, making it a high-performance, widely applicable micro-bioreactor. For example, Lewis et al. found that Ambr 15TM systems showed high consistency with 7 L reactors in terms of cell viability and protein productivity [12]. Hsu et al. compared growth performance of different cells in Ambr 15TM, 2 L reactors, and shake flasks, finding that all four CHO cell lines tested showed consistent results in Ambr 15TM with protein productivity differences within 13% [13]. Shahid et al. evaluated performance differences between Ambr 15TM, 3-15 L, and 200 L reactors for the same process, finding deviations of only 10-15% across scales [14].

2.3 Ambr 250TM

Ambr 250TM (Sartorius Stedim Biotech, UK) is an upgraded product following Ambr 15TM. As shown in Figure 5 [Figure 5: see original paper], its culture volume has been expanded to 200-250 mL, fully meeting purification and analytical requirements. The reactor geometry resembles traditional 1-3 L reactors, featuring a dual-layer pitched-blade impeller, L-shaped sparger with surface aeration, and four integrated baffles to enhance liquid mixing. Four additional side ports connect directly to the workstation for simultaneous feeding of multiple reagents. A single workstation can control 2, 4, 6···12, or 24 reactors in parallel, with each micro-bioreactor equipped with independent pH and DO electrodes, enabling individual control of process parameters for flexible experimental design. Ambr 250TM retains the fully automated operation mode combining robotic arms with workstations.

Research demonstrates that Ambr 250TM is an excellent high-throughput micro-bioreactor well-suited for clone screening and process optimization across different host systems, showing good consistency with large-scale reactors. Bareither et al. systematically compared Ambr 250TM with 3 L reactors, finding highly consistent performance for yeast, E. coli, and mammalian cells [15]. Rachel et al. evaluated consistency between Ambr 250TM, traditional 1-3 L reactors, and pilot-scale reactors, showing that CHO cells, yeast, and E. coli performed similarly in Ambr 250TM and large-scale reactors [16]. Xu Ping et al. used Ambr 250TM for CHO cell clone screening compared with 1-3 L reactors [17], demonstrating that scaling based on equal KLa enabled high consistency in cell culture between Ambr 250TM and 1-3 L reactors, with process development results successfully scalable to large-scale bioreactors. Additionally, Ambr 250TM can replace traditional small-scale reactors for process characterization during late-stage clinical studies. Mitchell et al. successfully combined Ambr 250TM with DoE using Definitive Screening Design (DSD) to complete late-stage process characterization for a fusion protein produced in E. coli, concluding that Ambr 250TM is a reliable scale-down model that can significantly shorten process characterization timelines when combined with effective DoE methods [18].

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3 Current Limitations and Future Directions of High-throughput Micro-bioreactors

The three common high-throughput bioreactor systems described above each have distinct features and demonstrate outstanding performance in various aspects of cell culture process development, yet they inevitably have limitations.

SimcellTM's notable features are ultra-micro volume and ultra-high throughput, but its shortcomings warrant attention. First, the 800 L culture volume cannot meet common offline analytical requirements [19]. Second, while the system can monitor cell density online, it cannot assess cell viability. Additionally, the lack of mechanical impellers prevents simulation of large-scale reactors, and its operating system is relatively complex with high initial capital investment.

Since becoming a research focus in 2010, Ambr 15TM has also reported several issues [20-22]. For instance, the 10-15 mL culture volume often yields insufficient samples for downstream process development; the stability of pH and DO sensor patches needs improvement; different temperature and agitation controls cannot be implemented within the same workstation. Due to the small volume, reactor characterization studies are inconvenient and can produce relatively large errors. For example, the power number initially provided by the manufacturer was 0.6, suggesting similarity to conventional reactors and supporting a P/V-based scale-up strategy. However, after Nienow et al. re-evaluated Ambr 15TM reactor characteristics in 2013 [21], the revised power number was 2.15. This discrepancy demonstrates that volume reduction actually increases the difficulty of understanding and controlling micro-bioreactors. Moreover, P/V values calculated from different power numbers vary significantly: at conventional speeds (900-1000 rpm), the P/V value is approximately 28 $W \cdot m^{3}$ with a power number of 0.6, but 100 $W \cdot m^{3}$ with the revised power number of 2.15, substantially challenging traditional scale-up approaches. Under equivalent cell performance conditions, achieving sufficient oxygen transfer coefficient (KLa) requires high agitation speeds, resulting in P/V values in Ambr 15TM more than ten times those in traditional 1-3 L reactors [21]. Overall, unlike traditional 1-3 L reactors, P/V can no longer serve as the basis for agitation speed setting in Ambr 15TM; instead, tip speed is used as the scale-up criterion [22].

Ambr 250TM, as the latest product, offers superior operability and closer approximation to large-scale bioreactors in geometric structure, hydrodynamic properties, and control strategies. However, practical applications are not without challenges. For example, the micro-control system requires extensive experimental tuning and refinement; pH and DO control often exhibit lag due to small volume; operational costs are relatively high, with each round consuming large quantities of single-use reactors and consumables, necessitating stricter R&D cost control. Compared to traditional reactors, balancing time and cost relationships is critical for new high-throughput micro-bioreactors, which must not only operate reliably and produce consistent results but also align with user objectives to deliver exceptional value.

Addressing these limitations, manufacturers can implement improvements through various measures. For example, developing micro-scale online/offline analytical methods to reduce sample consumption can overcome the sample volume limitations of SimcellTM and Ambr 15TM, maximizing their high-throughput advantages. As electrode membrane and optode technologies mature, sensitivity issues will be resolved. Manufacturers should actively follow up on practical applications, continuously summarize issues, make reasonable improvements, accumulate extensive data, and ensure excellent performance and consistency with large-scale bioreactors. Cost control also requires careful consideration to balance cost differences between micro and traditional reactors. Additionally, expanding application scope to better accommodate diverse culture modes is essential. For instance, our institution has successfully developed a perfusion culture simulation using Ambr 15TM for early-stage media screening in perfusion processes, greatly improving screening efficiency. Meanwhile, Sartorius Stedim Biotech has combined Ambr 250TM with hollow fiber cartridges to create the new Ambr 250 PerfusionTM product.

In summary, breakthroughs in detection technology, optimization of micro-bioreactor geometric models that meet scale-up principles, and continuous expansion of application scope—combined with reasonable protocols based on actual process development needs that balance time and cost—will enable high-throughput micro-bioreactors to maximize their advantages and become the mainstream trend in future cell culture process development.

High-throughput micro-bioreactors offer advantages of simple operation, high throughput, and cost reduction, significantly improving process development efficiency while lowering R&D costs and shortening timelines. These systems can be effectively combined with DoE for process development under Quality by Design (QbD) principles [23-24], enhancing process stability and quality controllability, which further broadens their application prospects.

Although current mainstream high-throughput micro-bioreactors have certain limitations, they essentially meet fundamental process development needs. Biopharmaceutical companies can leverage their advantages to make multi-factor, multi-level, large-scale experiments simple and efficient through the following strategies: (1) Integrate domain expertise and accumulated data with DoE for targeted, accelerated screening of optimal conditions [25-26]; (2) Based on reactor structural features and hydrodynamic characteristics, thoroughly evaluate consistency between micro and large-scale reactors to establish robust scale-up platforms; (3) Introduce Process Analytical Technology (PAT) during process development, combine multivariate analysis (MVA) based on batch models for systematic analysis of process parameters [27-28], and utilize high-throughput analytical techniques to predict metabolic changes [29-30] for better bioprocess control.

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5 References

[1] Reichert J. Antibodies to watch in 2015. MAbs, 2015, 7(1): 1–8.
[2] EvaluatePharma. World preview 2017, Outlook to 2022, the 10th edition. 2017: 1-41.
[3] Ecker DM, Jones SD, Levine HL. The therapeutic monoclonal antibody market. Mabs-Austin, 2015, 7(1): 9–14.
[4] Hay M, Thomas DW, Craighead JL, et al. Clinical development success rates for investigational drugs. Nature Biotechnology, 2014, 32(1): 40–51.
[5] Yao-ming Huang, Chris Kwiatkowski. The role of high-throughput minibioreactors in process development and process optimization for mammalian cell culture. Pharmaceutical Bioprocess, 2015, 3(6): 397–410.
[6] Legmann R, Schreyer H, Combs R, et al. A predictive high throughput scale-down model of mAb production in CHO cells. Biotechnology Bioengineering, 2009, 104(6): 1107–1120.
[7] Lamping S, Zhang H, Allen B, et al. Design of a prototype miniature bioreactor for high throughput automated processing. Chemical Engineering Science, 2003, 58(3):747–758.
[8] Isett K, George H, Herber W, et al. Twenty-four well plate miniature bioreactor high throughput system: assessment for microbial cultivation. Biotechnology Bioengineering, 2010, 98(5):1017–1028.
[9] Chen A, Chitta R, Chang D, et al. Twenty-four well plate miniature bioreactor system as a scale-down model for cell culture process development. Biotechnology Bioengineering, 2010, 102(1): 148–160.
[10] Rachel Legmann, H. Brett Schreyer, A. Peter Russo, et al. A Predictive High-Throughput Scale-Down Model of Monoclonal Antibody Production in CHO Cells. Biotechnology and Bioengineering, 2009, 104(6): 1107-1120.
[11] Amanullah A, Otero JM, Mikola M, et al. Novel micro-bioreactor high throughput technology for cell culture process development: Reproducibility and scalability assessment of fed-batch CHO cultures. Biotechnology Bioengineering, 2010, 106(1): 57–67.
[12] Lewis G, Lugg R, Lee K, et al. Novel automated microscale bioreactor technology: a qualitative and quantitative mimic for early process development. Bioprocess Journal, 2010, 9(1):
[13] Wei-Ting Hsu, Rigzen P. S. Aulakh, Donald L. Traul, et al. Advanced microscale bioreactor system: a representative scale-down model for bench-top bioreactors. Cytotechnology, 2012, 64(6):667–678.
[14] Shahid Rameez, Sigma S. Mostafa, Christopher Miller, et al. High-Throughput Miniaturized Bioreactors for Cell Culture Process Development: Reproducibility, Scalability, and Control. Biotechnology Progress, 2014, 30(3): 718-727.
[15] Bareither R, Bargh N, Oakeshott R, et al. Automated disposable small-scale bioreactor for high-throughput process development: implementation of the 24 bioreactor array. Pharmaceutical Bioprocessing, 2015, 3(3): 185-197.
[16] Rachel Bareither, Neil Bargh, Robert Oakeshott, et al. Automated Disposable Small Scale Reactor for High Throughput Bioprocess Development:

A Proof of Concept Study. Biotechnology and Bioengineering, 2013, 110(12): 3126-3138.
[17] Ping Xu, Colleen Clark, Charo Scott. Characterization of TAP Ambr 250 disposable bioreactors, as a reliable scale down model for biologics process development Biotechnology Progress, 2017, 33(2): 478-489.
[18] Mitchell Tai, Amanda Ly, Inne Leung, et al. Efficient High-Throughput Biological Process Characterization: Definitive Screening Design with the Ambr250 Bioreactor System. Biotechnology Progress, 2013, 31(5): 1388-1395.
[19] Vijay Janakiraman, Chris Kwiatkowski. Application of High-Throughput Mini-Bioreactor System for Systematic Scale-Down Modeling, Process Characterization, and Control Strategy Development. Biotechnology Progress, 2015, 31(6): 1623-1632.
[20] Moses S, Manahan M, Ambrogelly A, et al. Assessment of AMBRTM as a model for high-throughput cell culture process development strategy. Advance Bioscience Biotechnology, 2012, 3(7): 918–927.
[21] Nienow AW, Rielly CD, Brosnan K, et al. The physical characterisation of a microscale parallel bioreactor platform with an industrial CHO cell line expressing an IgG4. Biochemical Engineering Journal, 2013, 76(2): 25–36.
[22] Martin Paul Clarkson. The Ambr®15 Cell Culture User Manual. TAP-9670-06-005 Issue 7.30, 2016: 13-21.
[23] Rathore A. Implementation of quality by design (QbD) for biopharmaceutical products. Pharmaceutical Science Technology, 2010, 64(6): 495–496.
[24] Cogdill RP, Drennen JK. Risk-based Quality by Design (QbD): A Taguchi perspective on the assessment of product quality, and the quantitative linkage of drug product parameters and clinical performance. Journal of Pharmaceutical Innovation, 2008, 3(1): 23–29.
[25] Goldrick S, Holmes W, Bond NJ, et al. Advanced multivariate data analysis to determine the root cause of trisulfide bond formation in a novel antibody-peptide fusion. Biotechnology Bioengineering, 2017, 114(10): 2222-2234.
[26] Karst DJ, Scibona E, Villiger TK. Modulation and modeling of monoclonal antibody N-linked glycosylation in mammalian cell perfusion reactors. Biotechnology Bioengineering, 2017, 114(9):
[27] Lia Tescione, James Lambropoulos, Madhava Ram Paranandi, et al. Application of Bioreactor Design Principles and Multivariate Analysis for Development of Cell Culture Scale Down Model. Biotechnology and Bioengineering, 2015, 112(1): 84-97.
[28] Kwiatkowski C, Huang Y-M, Kshirsagar R, et al. Traversing six logs in scale-down modeling: two case studies in developing a scale-down model for the advanced microscale bioreactor system from a 15,000L production bioreactor. Presented at: 2013 AIChE Annual Meeting, San Francisco, CA, 2013: 3–8.
[29] Kelly W, Veigne S, Li X, et al. Optimizing performance of semi-continuous cell culture in an ambr15™ microbioreactor using dynamic flux balance modeling. Biotechnology Progress, 2017. DOI: 10.1002/btpr.2585.
[30] Shukla AA, Rameez S, Wolfe LS, et al. High-Throughput Process Development for Biopharmaceuticals. Advance Biochemical Engineering Biotechnology, 2017. DOI: 10.1007/10_2017_20.