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REVIEW

Developments and opportunities in continuous biopharmaceutical manufacturing

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ABSTRACT

Today's biologics manufacturing practices incur high costs to the drug makers, which can contribute to high prices for patients. Timely investment in the development and implementation of continuous biomanufacturing can increase the production of consistent-quality drugs at a lower cost and a faster pace, to meet growing demand. Efficient use of equipment, manufacturing footprint, and labor also offer the potential to improve drug accessibility. Although technological efforts enabling continuous biomanufacturing have commenced, challenges remain in the integration, monitoring, and control of traditionally segmented unit operations. Here, we discuss recent developments supporting the implementation of continuous biomanufacturing, along with their benefits.

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The current state of biopharmaceutical manufacturing

Most biopharmaceutical drugs are manufactured in batches in which human intervention is required to process a set quantity of material to be produced at the same time. Such operations were reasonable in the early phases of the industry, but are inefficient and may be unsustainable as the global demand for these drugs grows and the drug cost exceeds the purchasing power of an average individual.[1] An alternative manufacturing approach that relies less on human labor and transitioning steps between unit operations, requires a smaller facility footprint, and is more amenable to scaling, automation, and adaptation across different drug modalities, is desired. Continuous manufacturing is such an alternative that is gaining increasing popularity in the pharmaceutical industry. Continuous operation in the literal sense would proceed without pauses. In a more practical or transitional sense, a continuous process may include periodic or cyclic operations. Many industries, such as the chemical, petrochemical, food, and mechanical industries, have transitioned from batch to continuous manufacturing to lower costs while addressing growing demands.[2–4]

The pharmaceutical industry is among the most conservative sectors, due in large part to regulatory and product safety considerations, and innovation often occurs via mergers and acquisitions of smaller companies and start-ups.[5] Although the first recombinant biologic emerged in 1982, the biopharmaceutical industry has yet to manufacture affordable drugs at the maximum possible efficiency. Process innovation has been slow, partially stemming from limited drug-price regulation in the US. Given this autonomy and the high costs of other factors, such as clinical trials, in drug development, pharmaceutical companies have worked more to bring products to market quickly than to lower the cost of manufacturing.[6] To increase biologics affordability, both Europe and the US created approval pathways for biosimilars more than 10 years ago. Several dozen biosimilar products had been approved for use in those two jurisdictions as of mid-2020, but a general observation is that the savings afforded by these biosimilars have been relatively limited;[7–9] the economic incentive for biosimilar development in the US is "currently unstable".[10]

The cost of goods is not the main driver for biopharmaceutical prices, whether innovator or biosimilars, especially in the US. Nevertheless, the rising prospects of biosimilars and encouragement from the Food and Drug Administration (FDA) approvals of five small-molecule drugs manufactured with continuous elements between 2015–2017 have brought about a growing interest in continuous biomanufacturing[2] and the commercial launch of continuous unit operations. Apart from enabling the sustainable production of biosimilars, continuous biomanufacturing can also reduce the cost of innovator drugs and likely help address drug shortages.[6,11] Continuous manufacturing can also reduce the process footprint[12,13] and allow for greater domestic manufacturing capabilities.[2,14] The ability to manufacture most routinely prescribed drugs domestically in combination with reduced regulation has promoted competition and reduced the production cost of biologics in India and China to about 10% of that in developed countries.[1]

Acknowledging innovations in the manufacturing of small molecule drugs, regulatory agencies have urged the adoption of continuous biomanufacturing.[15] Although fully continuous bioprocessing has yet to be implemented commercially, as of 2015 perfusion cell culture and other continuous operations had been incorporated into the production of at least 19 commercial biologic products.[16] Despite these advances, several shortcomings remain, including technological gaps in process

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integration, real-time monitoring, and control, all of which would present challenges not just in commercial manufacturing, but also in process development. The lack of a precedent also raises concerns about regulatory uncertainty. Furthermore, investing in continuous biomanufacturing facilities without prior success also presents a business challenge.

Here we review recent efforts in continuous unit operations, integrated processes, and process analytical technologies (PATs) that facilitate the implementation of continuous biomanufacturing, particularly of monoclonal antibodies (mAbs). Apart from assessing the technical progress and remaining gaps related to continuous biomanufacturing, we discuss the associated business, regulatory and societal incentives and risks. Finally, we propose measures to address the major factors limiting the adoption of continuous biomanufacturing.

Continuous upstream processes

The enabling technology for continuous cell culture, a perfusion bioreactor, was introduced to increase cell titers.[17] Fresh media is fed and depleted media is removed at the same rate. The removed media is then routed to a cell retention device to extract the harvested cell-culture fluid and to recycle the cells back into the perfusion reactor. Additionally, a cell bleed is used to remove dead cells and improve cell viability. Given the continuous flow across a perfusion bioreactor, the cellular mean residence time is shorter and the distribution narrower,[12] rendering the product quality more consistent[18,19] than in batch and fed-batch bioreactors.

Apart from greater consistency in cell viability,[20] higher productivity[20,21] is seen in perfusion culture than in fed-batch. There is also less buildup of undesirable metabolites and other impurities.[20–22] The lack of a continuous stream across a fed-batch reactor translates to a product residence-time distribution (RTD) spanning from the beginning to the end of the cell-culture period.[12,22] Such a variable and lengthy product residence time inside a bioreactor may lead to undesirable product quality with respect to aggregation, fragmentation, chemical modifications, and post-translational modifications such as glycosylation.[20,21,23,24] Given the shorter product residence time inside a perfusion bioreactor, it has become a preferred cell-culture method for producing degradation-prone therapeutics.[12,25]

Given the benefits afforded by perfusion culture, this continuous upstream operation has been incorporated in many commercial processes. Between 1993 and 2003, 14 biopharmaceutical processes using perfusion cell culture were introduced, with an additional 8 between 2004 and 2014.[25] This decline in the growth of perfusion may be attributable to alternative routes to increased productivity such as improvements in cell-line engineering, media formulation,[26–28] and bioreactor design and control,[29] which increased the cell and product titers attainable via fed-batch technology from ~ 5 million cells/mL and 1–2 g/L to ~ 15 million cells/mL and 9–10 g/L in a 14–18 day culture, respectively.[30,31] Furthermore, the continuous feeding of fresh media and removal of spent media in a perfusion bioreactor necessitates a large volume of media and the associated storage and handling. However, media optimized and developed for perfusion, as opposed to commercial media in which the media composition is fixed, can reduce costs by allowing for a lower perfusion rate and requiring a smaller volume of media.[32,33] Media and perfusion rate optimization can add to the existing benefits of higher productivity, lower footprint, and the ability to integrate with downstream processes, to eventually reduce the higher cost[34,35] associated with perfusion processes.

The utility of a perfusion bioreactor extends beyond its use as a production bioreactor to its use in a perfusion-based high-density cell banking process,[36,37] which can reduce the number

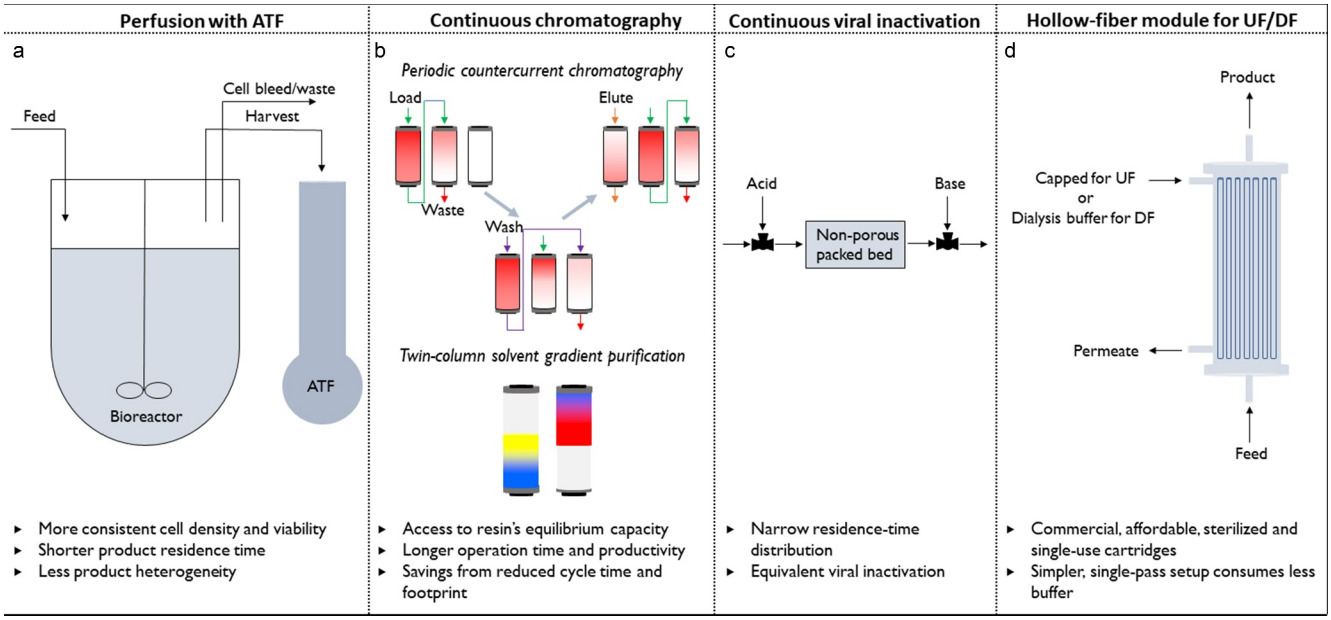

Figure 1. A subset of proposed technologies for continuous biopharmaceutical manufacturing. (a) Perfusion cell culture with alternating tangential flow filtration (ATF) for cellular retention, (b) 3-column periodic countercurrent chromatography for product capture[44] and a two-column solvent gradient purification method for product polishing,[45] (c) a continuous packed bed viral inactivator,[46,47] and (d) hollow fiber dialysis module for continuous ultrafiltration[48] and diafiltration.[49]

of expansion steps typically seen in the traditional seed-train. Furthermore, using a perfusion bioreactor in the N-1 stage of the seed train increases the inoculum density and shortens the culture growth phase.[38] Overall, the combination of a high-density cell bank, disposable Wave bioreactors and a perfusion N-1 culture shortens the seed-train expansion process while reducing manual operation and the risk of contamination.[38]

A critical enabling component in the operation of perfusion bioreactors is the cell retention device. The cell clarification may be carried out in the bioreactor using spin filters and submerged membrane filters or sequentially in a separate unit based on, e.g., filtration, centrifugation, or acoustic aggregation.[4] Spin filters have been used with perfusion mammalian cell culture for the industrial production of mAbs.[39] Although both submerged hollow-fiber filters and spin filters can be back-washed,[4] membrane fouling remains a challenge in their operation.[40,41] In fact, fouling of spin filters has prompted calls for modifications to the clarification process of an approved mAb.[39,42]

Cell retention may also be accomplished in a separate filtration unit, such as a tangential flow filtration (TFF) device. However, the recirculation of the sample through a peristaltic pump in TFF introduces shear damage and cell lysis.[43] This is ameliorated in alternating tangential flow filtration (ATF) (Figure 1a), in which the sample is pumped in alternating directions of flow over the membrane surface using a diaphragm pump. Although ATF is the most popular cell-retention technology, its cellular residence time must be considered in optimizing cell growth and productivity.[50] Membrane fouling must also be mitigated, as it decreases membrane permeability and filtrate flow. A continuous microfluidic device has resistance in the retentate flow and uses a variable sample flow rate to flush the membrane periodically.[51] The device boasts a small footprint, but requires further optimization, especially at a larger scale.

Another method for cell retention uses high-frequency resonant ultrasonic waves, instead of a membrane, to retain viable cells selectively from harvest cell-culture fluid from development to commercial scale.[52] Although acoustic devices have been combined with 200 L/day perfusion cell culture,[53] a significant loss of separation efficiency is seen upon further scale-up,[53] requiring a secondary clarification method such as depth filtration. Finally, continuous centrifugation can also be used. Although the continuous solids-discharge centrifuge clarifies harvest more efficiently than a periodic solids-discharge centrifuge,[54] scalability remains a challenge.[19,55] Complete clarification is also difficult to achieve with centrifugation, often requiring depth filtration for further clarification.[56]

Continuous downstream processes

Improvements in purification productivity have not kept up with the increase in product titer,[57] making downstream purification a potential bottleneck[58] in bioprocessing. Indeed, appreciable improvements in downstream productivity may be attained with a transition to continuous operations.[4,59] The typical platform purification process for the largest class of biologics, mAbs, consists of Protein A chromatography, a low-pH hold viral inactivation step, one or more polishing chromatography steps, a viral filtration step and an ultrafiltration/diafiltration step. These purification unit operations are carried out sequentially. Continuous purification would allow the processes to proceed uninterrupted.

The costliest unit operation in the purification of mAbs is Protein A affinity chromatography, which captures the product mAb from the cell-culture fluid while allowing other proteins to flow through the column unbound. The bound product is then eluted from the column using a low-pH buffer and kept in holding tanks for approximately an hour for viral inactivation. This batch operation of Protein A chromatography is unattractive on at least two counts. The first is low productivity due to the time spent in washing, eluting, and regenerating the column following sample loading. The second is the mass-transfer limitations that prevent use of the full capacity of the resin, as sample loading onto the column is stopped when the mAb breaks through, to avoid a loss of yield.

To overcome the low resin utilization, multicolumn processes such as periodic countercurrent chromatography (PCC) (Figure 1b),[44,60] sequential multicolumn chromatography[61] and simulated moving-bed chromatography (SMB)[62] capture the breakthrough exiting a column on another column. Two or more columns are then organized in loading and elution/regeneration phases to increase operating time and productivity. Compared to batch operation, significantly greater column productivity and resin utilization are seen even upon increasing the number of columns to two (Capture SMB)[63] for product titers greater than 2 mg/mL.[64] Compared to batch operation, the continuous multicolumn approaches require lower resin volumes and cycle times to produce the same amount and quality of the product,[65] offering savings.[66] Although scale-up from bench to pilot scale[67] and clinical manufacturing[68] has been demonstrated, implementation for commercial manufacturing is yet to be seen, primarily due to the complexities in the design and operation, involving many valves, automation and control, and the need for integration of PAT.[19]

The mass-transfer limitations of column chromatography may be reduced in part by using adsorptive membranes.[69] Higher flow rates are possible, reducing the purification time and improving productivity. Membranes for affinity-based capture of mAbs may incorporate ligands such as Protein A.[70,71] Although these membrane adsorbers do not have the capacity of packed-bed affinity chromatography, resulting in higher buffer requirements and higher process mass intensity,[13,72] this can be ameliorated when the adsorbers are configured for continuous capture. A continuous four-membrane adsorber periodic countercurrent chromatography system with increased throughput compared to Protein A column chromatography has been reported.[73] In addition, there have been considerable increases in capacity in newer generations of adsorptive membranes.

Although the familiar multicolumn chromatography set-ups are the leading choices for continuous product capture, non-column and non-chromatographic approaches have also been presented. Unlike the multicolumn methods, the column-free continuous countercurrent tangential chromatography (CCTC) is operated at steady state. CCTC incorporates static mixers that improve the rate of contacting of the resin slurry

and the cell-culture fluid, and hollow-fiber membrane modules that are permeable to the products and the impurities, but not the resin beads.[74] The retentate and permeate move countercurrently and recycling is incorporated. This continuous operation is presumably easier to integrate with perfusion cell culture and showed comparable yield and purity to that of a batch column operation, but with higher productivity.[75] Although residence times of as little as 7–12 min are necessary for capture in CCTC, compared to ≥ 1 hr for a Protein A column,[75] the slurry-based operation results in more dilution than in a column and consequently requires a larger buffer volume. Despite recent efforts[76] to address such issues, this technology requires further maturation for industrial adoption and long-term continuous processing.

Although adsorptive methods prevail, product may also be captured by aqueous two-phase extraction (ATPE), a liquid-liquid fractionation technique that leverages the phase separation driven by two immiscible polymers or a polymer and a salt.[77] Affinity ligands may also be incorporated into ATPE to improve the selectivity of the process.[78] Such an approach at preparative scale can boast a high ligand concentration per volume of the polymer solution, allowing for higher productivity.[79] Although numerous applications of ATPE have been demonstrated over several decades,[80] a continuous pilot[81] and mini-plant-scale[82] ATPE system for mAb purification was demonstrated more recently. Efforts to integrate aqueous two-phase systems with membranes[83] and traditional platform purification processes[84] have resulted in mAb yields between 78–74%, with no changes in the glycosylation profile.[84] Additional process maturation and improvements in yield may be needed to enable large-scale adoption of ATPE in biomanufacturing.

Following mAb capture, viral inactivation is typically carried out in batch processing by holding the low-pH Protein A eluate in a large tank for approximately 1 hr. This can also be performed continuously, as shown recently using a continuous reactor with a narrow RTD, where significant viral inactivation was observed after just 15 mins.[46] To ensure an appropriate minimum residence time, the FDA recommends evaluating the RTD for continuous viral inactivation.[85] To this end, three viral inactivation reactor designs have been proposed: a coiled flow inverter,[86,87] a tubular reactor called jig-in-a-box,[88,89] and a packed-bed reactor[46,47] (Figure 1c). Radial mixing is enhanced in the first two designs due to the presence of helical structures and alternating 270° turns, respectively. In the packed-bed reactor, nonporous particles ensure a narrow RTD. Collectively, these advances demonstrate that viral inactivation may be adapted for continuous manufacturing. However, challenges remain to be overcome in the integration of continuous viral inactivation reactors with multicolumn chromatography methods that process sample periodically.[46] The variation in the pH and concentration of the affinity eluate over time may also hinder the performance of continuous viral inactivation, requiring an additional hold step.[46] In addition to viral inactivation, viral filtration may also be carried out continuously[90] under a lower pressure over an extended duration. Filters appropriate for such an operation must be carefully chosen,[90] and multiple filter set-ups may be considered.

Polishing steps that are performed using bind-and-elute chromatography may be operated continuously using approaches similar to those discussed for capture chromatography earlier. The dominant methods are the multicolumn chromatography approaches such as SMB and multicolumn countercurrent solvent gradient purification (MCSGP) (Figure 1b). These methods overcome the purity-yield trade-off that exists in batch chromatography through internal recycling of the overlapping region in the chromatogram.[91] SMB processes do not permit linear gradients and are preferred for size-exclusion, hydrophobic-interaction, and mixed-mode chromatography operations.[92] A comparison between a capture SMB and a single column operation revealed similar viral clearance despite a difference in the residence time and loading amount.[93]

Compared to isocratic SMB processes, MCSGP combines the continuous countercurrent migration with a solvent gradient to separate mixtures of more than two components, making it suitable for continuous ion-exchange chromatography.[94] Internal recycling is leveraged during elution in the polishing processes, as opposed to during loading, as is done in the capture processes.[91] MCSGP has been employed in the separation of mAb charge variants,[45] aggregates and fragments.[95]

A possible approach to bind-and-elute mode in polishing chromatography is to perform frontal loading, in which the column is overloaded, initiating competitive binding and subsequent displacement of more weakly bound proteins by competitor species with a stronger affinity to the resin. As such, significant separation is achieved during sample loading in addition to that during elution. This approach can be particularly effective if the impurity binds more strongly than the product, as is often the case for aggregates and basic impurities vs. mAbs on cation-exchange resins. The weaker binding of the product and the shorter residence time on the column in frontal operation can also mitigate potential on-column unfolding.[96,97] Despite these benefits, frontal chromatography has not been implemented widely in bioprocessing due to concerns regarding process robustness.[98] However, it is reemerging[98-101] amidst process intensification efforts as it can increase polishing productivity and decrease the manufacturing footprint, and it can also be implemented in continuous processing. Recently a continuous two-column countercurrent frontal chromatography process was presented for the separation of mAb aggregates from monomers.[100] In addition, multicolumn chromatography approaches leveraging displacement among the product and impurities such as aggregates, host-cell proteins, and charge variants have been proposed;[99,101] these methods can be adapted to be continuous.

Under conditions in which the product does not bind to the resin, the impurities that bind can be separated using flow-through chromatography. Given the low levels of impurities generally seen in the polishing stage of purification, flow-through separation can increase productivity by reducing the required amounts of resin and media. Furthermore, the lack of a product elution step allows continuous product flow. Two or more flow-through unit operations have been integrated for the polishing[102,103] and complete purification[104] of antibodies. Despite these developments, the complexities in the design of

multicolumn polishing systems are still disincentives for manufacturing implementation.[19] Process modeling and simulations can alleviate the perceived risks, as they can assist process development and optimization,[99,100,105] along with experiments. Single-use technologies such as membrane adsorbers[106-108] are also suitable for flow-through applications, which require less adsorption capacity. A continuous purification train using only commercial membrane adsorbers enables higher flow rates and greater productivity, making it particularly suitable for clinical production.[109]

The chromatography steps in a typical downstream process typically receive most attention because they are capable of highly selective separations, but they are simultaneously most challenging to convert to continuous operation. A necessary adjunct, though, is the need for buffer exchange and/or product concentration, usually to prepare the eluate of one chromatography step for loading onto the next step. This can be achieved using ultrafiltration/diafiltration (UF/DF) operations that are readily amenable to continuous operation. TFF implemented with a single pass (SPTFF) can be operated continuously and has been used for inline concentration of products between unit operations across the process.[110,111] However, continuous UF/DF requires more than one SPTFF module in sequence to concentrate and exchange buffer. Recently a pilot-scale continuous process with a 3-stage SPTFF was presented. The sequential arrangement of the SPTFF units allowed for rounds of volume reduction followed by dilution with the diafiltration buffer, resulting in buffer exchange of > 99.75%.[112] Operating such a membrane module in countercurrent mode reduces the buffer requirement.[113] Such desalting and buffer-exchange operations can be performed with savings in cost, buffer and operational complexity by using presterilized hollow-fiber cartridges, which have a shorter path length and larger surface area, and require a lower feed flux[48,49] (Figure 1d). Continuous and single-use protein concentration (10x)[49] and buffer exchange (99.9%)[48] with the hollow-fiber systems have been demonstrated and can be combined for integration into continuous biomanufacturing.

To avoid using multiple SPTFF units, a preliminary design of a 3D-printed two-membrane set-up that achieves UF/DF simultaneously was proposed recently.[114] The design confines the feed flow between two membranes and the pressure differential above and below the feed flow affords simultaneous concentration (4.5x) and salt reduction (47%).[114] The authors state that additional efforts in size reduction, parallelization, and simplified hydraulics of the set-up are necessary to advance the tool.

Continuous formulation processes

Biopharmaceuticals are formulated into high-concentration liquid or lyophilized solid formulations; approximately one-third of successful mAb formulations are lyophilized, while the remainder are liquids of concentration 2–200 mg/mL.[115] Liquid formulations are preferred over lyophilized formulations for stable products because they are more straightforward to produce and administer.[115] The UF/DF strategies discussed in the previous section can enable continuous product concentration and exchange into the desired buffer for formulation and purification, and the intrinsic suitability of membrane processes for continuous operation makes formulation, at least of liquids, especially amenable to adaptation from batch processing.

End-to-end integration

Continuous biopharmaceutical manufacturing requires end-to-end integration of all the unit operations, which represents a major challenge in establishing reliable continuous processes. The integration must accommodate continuous flow from one unit operation to another, leading to higher productivity. Such smooth operation requires understanding and synchronization of RTDs, flow rates and propagation of their disturbances across the production process. To this end, the need for an RTD model-building platform for a continuous bioprocess has been recognized.[116] Furthermore, if a key unit operation or its subunit fails, the ability to redirect the flow of the process stream through redundancy and parallelization may be necessary.[6,19] To this end, automated process-control strategies based on modeling techniques and sensitive real-time sensor technologies are desired. Global control strategies that enable feedforward and feedback control can mitigate the risks associated with process integration and continuous operation.

Perfusion cell culture has been successfully combined with continuous capture units at various scales. These include the integration of a perfusion bioreactor with a membrane chromatography unit,[117] a four-column (PCC) system[118] and a two-column countercurrent chromatography unit.[60] Another system integrated a perfusion bioreactor with two PCC systems for both continuous capture and successive polishing.[119] On the other hand, integration of downstream unit operations starting from product capture to formulation has also been demonstrated in a single-use downstream GMP process. Comparable and reproducible product quality with a productivity boost of 400–500% was reported.[120] Recently, a fully integrated pilot-scale continuous process from bioreactor through formulation based on single-use technologies demonstrated 4.6-fold greater productivity with a 15% cost reduction[59] compared to a batch process.

Process analytical technologies

An integrated process requires adequate PAT to monitor the critical quality attributes (CQAs), and indeed the FDA encourages the adoption of PAT to ensure product quality.[121] Doing so requires monitoring of critical process parameters (CPPs) and use of the knowledge behind how the CPPs affect the CQAs in process control strategies. The implementation of PAT is not trivial, given the complexity associated with a cellular host.

How individual CPPs affect different CQAs is an integral part of the Quality by Design framework even for batch operations, but in continuous processing such understanding must also be implemented for real-time process control during production. In addition, the understanding must be sufficiently broad to inform how any change will propagate further downstream due to the complete integration of disparate unit operations. Therefore, mature batch processes, especially platform

processes in which the CPPs and their effects have been tested on multiple mAbs, are good candidates for adaptation to continuous processing. Causal relationships are commonly developed empirically within a response-surface methodology approach, but there is also an ongoing effort to expand the availability of first principles-based mechanistic models, mainly at the level of individual unit operations. Although mechanistic models are often empirically parameterized,[122] they are preferred over fully empirical models. Although acceptable mechanistic models exist for certain unit operations, first principles-based understanding for the entire production train has not been achieved.[123] Upstream systems are especially challenging because of the hierarchy of levels of analysis that can be considered.

The quest for process intensification also supports alternative approaches such as "grey-box" modeling,[123] which incorporates fundamental principles found in mechanistic models with statistical correlations, especially given the large repositories of data for current processes available within companies. Studies using machine-learning models in the realm of pharmaceutical production are growing, with applications in predicting metabolite production,[124] biophysical properties,[125–127] chromatography modeling,[128] as well as in analytical data analysis,[129] sensor development,[130] and quality evaluation.[131] These efforts can help enable more broadly integrated models for both process development and real-time control in continuous biomanufacturing.

Continuous operation will also amplify the need for convenient and reliable instrumentation for inline monitoring. The parameters routinely monitored in batch operations, such as dissolved oxygen, flow rate, pressure, turbidity, pH, conductivity, and ultraviolet (UV) light absorbance, are necessary but not sufficient in the quality decision-making process. Key cell-culture process parameters have been monitored using spectroscopic methods such as near-infrared (NIR),[132] mid-infrared,[133] and Raman.[134,135] Characterization of glycoforms, charge isoforms, and host-cell protein levels are often determined off-line in a nonperiodic manner,[123] so alternative approaches, such as discussed below, may be needed.

For product characterization during purification, at-line analytical liquid chromatography systems often take as long as 30 mins[136] or operate under too high a pressure for convenient real-time process measurements. Although multi-wavelength spectroscopy with multivariate data analysis has been suggested for monitoring product concentration[137] or quantifying impurity concentrations in process streams,[138,139] opportunities for improvements in their sensitivity remain. More extensive assessment of CQAs will require more extensive measurements. Recent efforts include: 1) a combination of refractive index and multiple spectroscopic detectors,[140] analyzed using a multivariate partial least squares regression model; 2) label-free inline detection of product aggregates using hydrogel-encapsulated NIR fluorescence nanosensors[141] and of protein impurities using sensitive and inexpensive silicon nanowire biosensors;[142] 3) a continuous-flow nanofluidic device[143] for the measurement of macromolecular size, folding, and binding activity; 4) and a low-cost aptamer-based molecular turn-on assay that also monitors mAb concentration in real-time, with the ability to distinguish between native and heat-treated mAb.[144] Such innovations in sensor development are necessary not only for implementation in continuous manufacturing, but also to generate large data sets needed to improve process understanding.

Robust continuous operation will require coupling of measurements to control systems, with modeling used for both state estimation and control implementation. Data sets acquired over different time ranges pose additional challenges for adaptation to the real-time process monitoring and control that are desired for continuous manufacturing. Process parameters monitored in real time may be fed to models, empirical or otherwise, to predict key CQAs such as the glycosylation pattern[145-147] during the cell culture process. For example, glycosylation may be modulated through parameters such as amino acids,[148] manganese,[23,149] ammonia,[150–152] glycosylation precursors,[153,154] and other additives[146,155] in the cell culture media. Control strategies implemented in cell culture include the use of dielectric spectroscopy[156] and focused-beam reflectance[157] probes for viable cell-density measurement. In purification, many use UV-based control strategies for loading onto continuous chromatography set-ups.[60,158,159] Recently, a NIR flow cell has been integrated with a continuous SMB system for monitoring and control of mAb loading for product capture.[160]

Extension of process monitoring and control across the entire manufacturing train is critical to ensure successful continuous operation. For complete process integration, global control strategies should be implemented to assess the impact of upstream process conditions on downstream productivity, requiring elimination of the dichotomy between upstream and downstream processes. For example, upstream processes are currently optimized for higher titer and product quality, while downstream processes consider the removal of lumped measures of aggregates, charge variants and host-cell proteins. Changes in purification processes can alter the content and profile of impurities and product variants, making it desirable to control their generation early in the process. For example, media components[161] and harvest operations[162] have been found to influence the heterogeneities in the impurity[163] and product profiles.[164] Furthermore, inline monitoring of mAb aggregation in cell culture were demonstrated[165,166] to provide relief to purification efforts and improve process yield. Understanding the effect of operational parameters on the final product quality can be leveraged in controlling the process and meeting a target requirement, for example in the case of biosimilars.[12]

Advantages and disadvantages of continuous biomanufacturing

The multifaceted advantages of continuous biopharmaceutical manufacturing are summarized in Figure 2. A model comparison of a continuous bioprocessing platform with stainless-steel and single-use batch processes across clinical and commercial scales suggests that continuous operation can boost the savings afforded by single-use technologies.[167] The reduction in long-term production costs can make the pursuit of biosimilars economically more attractive for drug companies, improving the affordability of biologics.

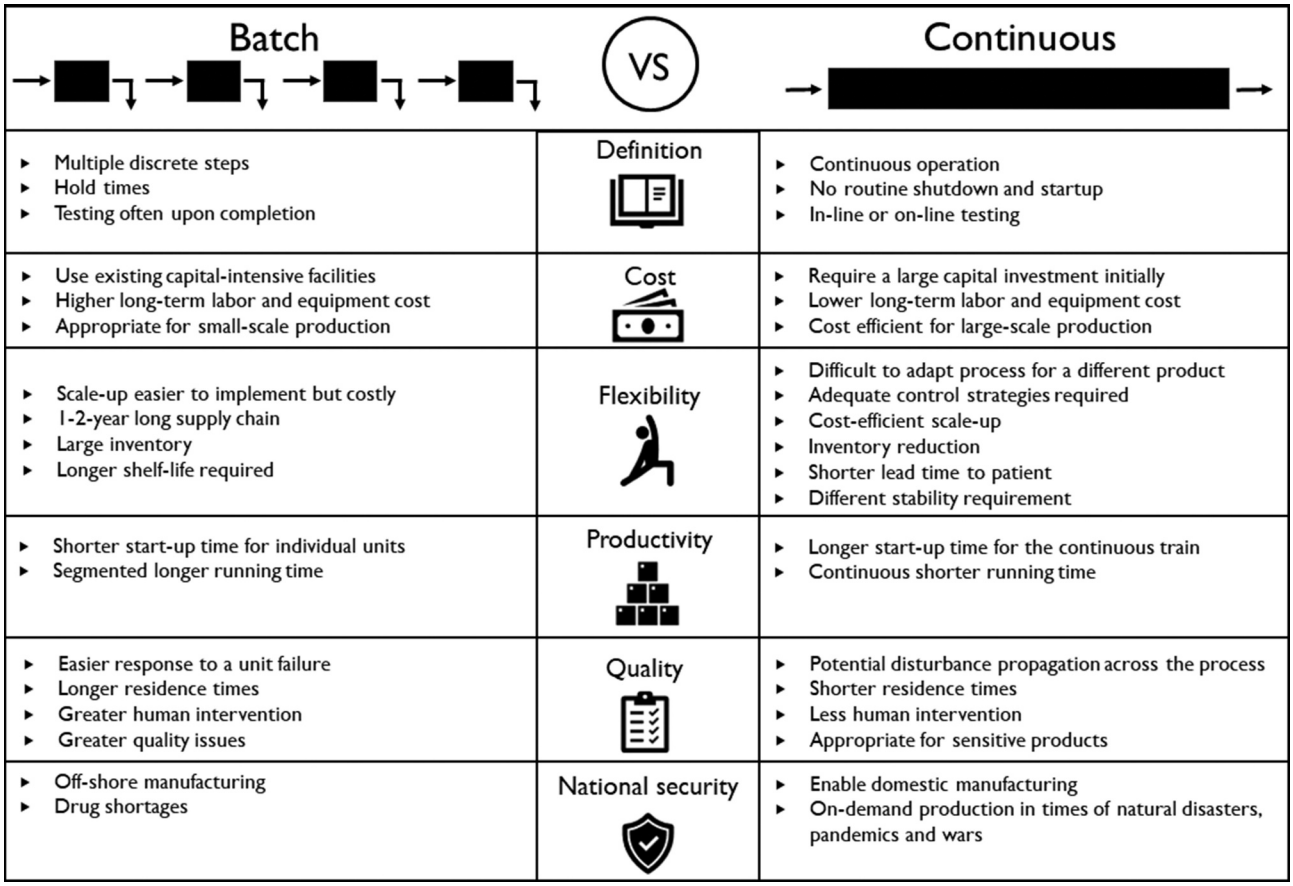

Figure 2. A comparison of batch and continuous pharmaceutical manufacturing.

In addition to affordability, continuous manufacturing can also improve the accessibility of drugs.[11] Failures in manufacturing quality can lead to drug shortages,[6,11] and continuous manufacturing can deliver products of consistent quality. The ability to produce potentially higher-quality drugs on demand can circumvent overproduction and delays.[168] Current batch manufacturing takes 1–2 years for the product to reach the customer, requiring a large and costly inventory.[168] Continuous manufacturing can also help facilitate more domestic manufacturing[169] of essential drugs, which can improve national security and increase drug accessibility in emergencies such as natural disasters, pandemics and wars. Currently, 80% of active pharmaceutical ingredients prescribed in the US are made abroad,[170] and many biologics plants are based in foreign countries due to a large footprint requirement, environmental liabilities, and lower labor costs. Continuous manufacturing can provide incentives for "back-shoring" of the offshored plants, as it requires a smaller footprint and relies less on labor than batch manufacturing.[2,169]

Several hurdles must be overcome to realize the advantages of continuous biomanufacturing. Although several continuous alternatives for batch processes have been demonstrated, the continuous integration of these unit operations is not trivial, particularly when the product outflow from some unit operations is cyclic. Successful process integration will require not only a better process understanding, but also the implementation and integration of PAT across unit operations. Such a novel endeavor requires risk-taking in both financial and regulatory filing aspects.

Conclusions

Pharmaceutical companies as well as academic, vendor, and government laboratories have commenced efforts to advance the continuous manufacturing of biopharmaceuticals to reduce costs and increase productivity, efficiency, drug accessibility, and national security. Several commercially approved processes already use continuous perfusion cell culture, but continuous downstream technologies have not yet been implemented in approved processes. While multicolumn recycling approaches are not truly continuous, they can provide higher productivity, smaller footprints, and cost savings compared to batch processing for large-scale production and have been integrated with cell-culture processes at various scales.

Process integration remains a primary challenge, for which advances in and integration of global PAT across the process are desired. Prior to the implementation of process control strategies, the development of continuous processes will require improved process understanding and less reliance on empirical know-how than for a batch process.[15] To this end, computational approaches can augment the understanding obtained from experimental methods and assist in the integration of processes beyond the mAb platform process to those of

emerging biologics, such as virus-like particles, exosomes, and gene and allogeneic cell therapeutics.

Improved process understanding, whether empirical or acquired from computational modeling, may be applied to process control for the automation of continuous manufacturing. The ideal operation of a manufacturing plant, with minimal human intervention, involves the integration of hardware and software, automated data analytics, process modeling, and fast inline or online sensors. Such a vision requires the recruitment of data scientists, process control engineers, systems biologists, and innovators in the field of sensors, among others, to address current biomanufacturing challenges. Additional fundamental research expanding our understanding of the impact of CPPs on CQAs will also strengthen the implementation of PAT.

The development and implementation of continuous biomanufacturing require a substantial initial investment. Once the initial capital investment of continuous manufacturing pays off, long-term benefits can be reaped,[167] including lower production costs due to a smaller equipment footprint[4,13] and reduced labor costs;[171] higher productivity due to operation over longer durations with no hold steps;[59,171] improved product quality due to a shorter residence time[7,23,25,,47] and benefits of model predictive control strategies.[12,172] Pharmaceutical companies may be reticent to invest fully in a yet-to-be commercially implemented endeavor, but, once a precedent for continuous biomanufacturing has been set, the business need for radically improved manufacturing should supersede their comfort with the current standards.

In addition to the financial risks and the technical gaps outlined here, regulatory uncertainties associated with the lack of prior approval are challenges facing the commercial adoption of continuous biomanufacturing. Recognizing that transitioning from the existing regulatory framework to new technologies can be a hurdle, the regulatory agencies have initiated measures to assist[6,173] with challenges before regulatory submission and to further support[174] innovation in drug development. In addition to the regulatory support, acceleration of process intensification may require additional immediate regulatory incentives apart from the much-evaluated long-term benefits. Incentives such as expedited approval, patent exclusivity period and tax reduction may fast-track the adoption of continuous biomanufacturing.[2]

Abbreviations

ATF, alternating tangential flow filtration; ATPE, aqueous two-phase extraction; CCTC, continuous countercurrent tangential chromatography; CPP, critical process parameter; CQA, critical quality attribute; FDA, Food and Drug Administration; mAb, monoclonal antibody; MCSGP, multicolumn countercurrent solvent gradient purification; NIR, near-infrared spectroscopy; PAT, process analytical technology; PCC, periodic countercurrent chromatography; RTD, residence-time distribution; SMB, simulated moving-bed chromatography; SPTFF, single-pass tangential flow filtration; TFF, tangential flow filtration; UF/DF, ultrafiltration/diafiltration

Disclosure statement

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