Single-Use Avoids a Midlife Crisis

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Single-Use Technology Is Consolidating Its Gains and Finding New Ways of Fitting In

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In its youth, before it joined the grown-up bioprocessing world, single-use technology was preoccupied with showing what it was made of—plastic—while defying conventions set by glass and stainless-steel systems. Now single-use technology is reaching middle age, and it is less interested in testing its strength and shaking up the status quo. Instead, single-use technology is consolidating its gains and finding new ways of fitting in.

Aware of its inherent limitations and, possibly, nursing regrets over its unrealized ambitions, single-use bioprocessing has all the excuses it needs to justify a midlife crisis. It could, for example, withdraw from challenging relationships and refuse to support interchangeable connectors and interoperability standards.

Fortunately, single-use bioprocessing is not so insecure. It is participating in hybrid systems at both the operational and process levels. It is also embracing appropriately supportive roles in single-use fermentation, adherent cell culture, and continuous processing.

Fermentation Bioreactors

Figure 1. Eppendorf's BioBLU single-use bioreactors are designed to extend the applicability of disposable processing to bench scale and lower.

Warming the heart of any lab scientist who has ever "done" his or her own glassware, single-use technology has settled into the small culture market. To help single-use technology establish itself in this sector, Eppendorf expanded its line of single-use fermentation bioreactors through the introduction of the BioBLU® 3f, an instrument that has working volumes of 1.25 to 3.75 L. The product, which appeared in 2016, extends the range of operation of disposable mini bioreactors through bench scale (*Figure 1*).
BioBLU 3f vessels employ robust overhead magnetic stirring at agitation rates up to 1,200 rpm, and incorporate submerged interior baffles for improved oxygen transfer. Eppendorf has demonstrated comparability between BioBLU f and autoclavable glass vessels, which BioBLU are meant to replace.

BioBLU vessels are made of a single-layer polymer that does not require additives or softeners, which mitigates issues related to leachables and extractables. "The vessels are very stable and the potential for vessel damage is reduced, compared to a bag design," says Karl Rix, Ph.D., head of the bioprocessing business unit at Eppendorf.

Importantly, the BioBLU 0.3 and BioBLU 1 vessels, which have working volumes of up to 250 mL and 1.25 L, respectively, are compatible with the DASGIP Parallel Bioreactor System and the DASbox Mini Bioreactor System for parallel control of up to 24 bioreactors.

The geometries of the differently sized BioBLU vessels are comparable to those of conventional reusable bioreactors/fermentors. At these volumes, single-use bioreactors are often used for process development, design-of-experiment studies, and scale-down/troubleshooting. "Designing a durable, single-use vessel for fermentation was technically quite challenging," notes Dr. Rix. "Microbial cultures grow much faster and to a much higher density than mammalian cultures, and therefore demand a more efficient supply of oxygen and nutrients. Furthermore, much more heat is released in microbial fermentation, which must be removed to keep the temperature at setpoint."

These requirements lead to specific differences in bioreactor design. Impellers for fermentations must run faster. For example, the BioBLU 0.3c vessel for cell culture applications runs at around 500 rpm, while the fermentation version BioBLU 0.3f agitates at up to 2,000 rpm. Blades are shaped differently as well.

"We use Rushton-type impellers (six rectangular square blades) for fermentation and pitched-blade impellers (tilted blades) for cell culture," Dr. Rix tells *GEN*. "The Rushton-type impellers achieve the efficient mass transfer needed in microbial processes, whereas the pitched-blade impellers cause less shear-stress and are therefore suitable for the cultivation of shear-sensitive mammalian cells."

Perfusion Bioreactors

Figure 2. PerfuseCell asserts that its CellMembra system qualifies as a single-use, scalable bio-reactor for adherent cell culture.

Single-use technology has become something of an enabler for continuous processing. According to serial entrepreneur Per Stobbe, CEO of Switzerland-based Stobbe Pharma Technology (SPT), what has been missing is a completely preassembled, single-use, ready-to-use perfusion bioreactor.

The solution comes from PerfuseCell, an SPT daughter company that has two perfusion cell culture products, CellMembra and CellRetention. The CellMembra system is built around a scalable, single-use, rigid polycarbonate bioreactor (*Figure 2*). Sized at 500 mL and up, the fully assembled system incorporates crossflow filtration and a single-use diaphragm pump for perfusion processes.

CellMembra is a configurable system that is optimized for research and development. The system's single-use pump removes process fluid from the bioreactor and forces it along the hollow fibers of the crossflow filter. Permeate passes through the filter membrane, and retentate returns to the single-use bioreactor. Preinstalled single-use sensors for dissolved oxygen and pH are included, with an optional single-use biomass sensor.
The fully scalable, similarly equipped CellRetention system will debut in 2018. Like the CellMembra system, it features single-use bioreactors equipped with single-use sensors for pH, dissolved oxygen, and biomass. In both systems, the installed diaphragm pumps are computer controlled.

CellMembra's future iterations, Stobbe expects, will operate at up to 1,000 L, depending on customer needs. "We control the pump and the drive systems, so there should be no size limit," Stobbe says, adding "but a 1,000-L perfusion system is huge."
PerfuseCell's systems fill gaps in existing single-use perfusion products, specifically the lack of an integrated single-use bioreactor. "In that regard," Stobbe asserts, "we are some years ahead of our competitors."

Adherent Cell Culture Systems

The major players in single-use suspension cell culture have been slugging it out over biobag size, materials of construction, scalability, hybrid systems, etc. At the same time, disposables have quietly taken over adherent cell culture, particularly at development and discovery scales. Corning is known for its stackable systems. Its CellSTACK® system for adherent cell culture is available in a one-stack configuration with a 636 cm^2 cell growth area. CellSTACK can also scale up to a 40-stack deployment with a 25,440 cm^2 cell growth area.

CellSTACK and its assembled Corning CellCube® products are targeted to vaccine and cell therapy processes for which the company's tagline "End-to-End Solutions for Scaling-up" makes sense. Corning also offers a portfolio of single-use products for suspension cell culture, most prominently disposable spinner and Erlenmeyer flasks. Corning also offers HyperSTACK®, which offers improved gas permeability and a culture area as high as 18,000 cm^2.

Corning leads the market in disposable Erlenmeyer-type vessels, according to Chris Mach, the company's director of bioprocessing. The flasks serve in seed train applications for large-scale commercial suspension culture-based production.

"We're in that market but only at the front end of the workflow," says Mach, "whereas with adherent cultures, we are present throughout the chain of expansion for biologics or vaccines, up to cell harvest."

Mach asserts that the question of single-use vs. autoclavable culture materials among Corning's customers has become moot: "We don't get much demand for anything reusable anymore, so all new products are specifically geared toward the single-use market. We've already gone past the volumetric needs for many customers in markets in which we participate, and all of them are asking for disposables."

Microcarriers, which Corning also sells (including a dissolvable bead product), allow scaling up to the typical high end for vaccine and cell therapy production needs, which is about 200 L.

Processes of 500 L or higher, which are common for most economically significant therapeutic proteins, are rare in these markets. At critical vaccine/cell therapy volumes, manufacturers still enjoy the option, with microcarrier-based adherent cell processes, to use single-use or clean-in-place bioreactors, or even hybrid systems.

Hybrid Operations

Figure 3. The Quattroflow 1200 bioprocess pump from Holland Applied Technologies is designed to provide drop-in replacement for conventional peristaltic pumps, with all-disposable process fluid contact.

In the past, manufacturing processes for biotherapeutics were conducted mostly (or entirely) with stainless-steel systems, or mostly with single-use systems. “What we see today is more hybrid situations,” says Danny Lyons, sales engineer at Holland Applied Technologies. “A lot of processes still use stainless-steel equipment (for example pumps, homogenizers, and other large equipment) and employ single-use when possible. But stainless steel is here to stay.”

Hybrid manufacturing can exist within a single unit operation. For example, a unit operation can integrate single-use nozzles and fill needles in an otherwise clean-in-place filling machine. More common are processes that use single-use bags for buffer “prep and hold” operations, which feed into stainless-steel bioreactors.

“Generally, each unit op tends to be either stainless or disposable,” Lyons adds. Fluid-transfer equipment is often specified as single use, as are sanitary connectors between single-use process steps or between disposable and stainless-steel operations.

Among Holland’s hybrid systems is the Quattroflow pump, a four-piston diaphragm pump that offers the option of using single-use process fluid contact surfaces (*Figure 3*). The Quattroflow 150, 1200, and 4400 models combine a polypropylene pump head with the standard USP class VI elastomers. “A lot of our customers want to have both options,” Lyons notes.

In the early days of single-use bioprocessing, components tended to be proprietary: Company X would guarantee a fluid transfer system only if it used its own connectors. “The problem is that once a single-use component is validated, customers will continue to request that specific product and overlook other options.”

Today, universal or interchangeable connectors are on the rise. With no plateau in sight for single-use bioprocessing, there will be greater incentives to make equipment that’s easy to integrate. “It becomes a source of competition for suppliers,” Lyons maintains. “Companies will have much more difficulty telling customers, ‘This is the only connector our equipment will work with.’”

Hybrid Processes

Figure 4. Avid Bioservices has been an early adopter of single-use technology, around which the company’s Myford, CA, facility was designed.

Avid Bioservices announced late last year that it had installed two of MilliporeSigma's Mobius® 2,000-L single-use bioreactors at Avid's clinical and commercial manufacturing facility in Myford, CA (*Figure 4*). The bioreactors satisfy growing demand larger single-use processes. The facility's flexible, modular design will allow the addition of a third 2,000-L bioreactor.

Gene Yoshioka, senior director of manufacturing at Avid, points to the trend among his company's clients for single-use technologies: "Company strategies now include contingencies to expand capacities quickly or transfer them from one site to another, which is only achievable through rapidly replicated single-use systems. While some legacy processes continue to require the use of stainless-steel systems, we work with our clients to determine the best development pathway to success for their molecule, whether that means continuing forward in stainless steel or transitioning to single use."

Toward that end, Avid maintains stainless-steel capacity at the 100-L, 300-L, and 1,000-L scales.

Ten years ago, facilities for high-volume biologic drugs were still designed around a specific process, at scales well beyond 2,000 L, making them very costly investments. With the induction of single-use technologies and the emergence of high-titer processes, therapeutic biotech has shifted its manufacturing in more flexible facilities that can be easily outfitted with multiple, smaller bioreactors. Today, 1,000- and 2,000-L single-use bioreactors are considered standard volumes to support commercial needs.

"Even before Avid commissioned its new facility 2016, it had experience incorporating disposable technologies through both upstream and downstream processes," Yoshioka explains. "As interest in single-use technologies accelerated, Avid became an early adopter."

Yet, disposable downstream technologies have had to play catch-up to advances made upstream. Single-use chromatography columns are just now being scaled to match the high titers that are prevalent today, and bioprocess skids with single-use flow paths are still limited by relatively low flow rates. "To offset these limitations," Yoshioka notes, "the industry is exploring continuous processing to keep equipment sizes small while still handling large amounts of product."

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