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Cover Design by Dan Ward
Images: Sebastian Kaulitzki - stock.adobe.com

BioPharm International is selectively abstracted or indexed in: • Biological Sciences Database (Cambridge Scientific Abstracts) • Biotechnology and Bioengineering Database (Cambridge Scientific Abstracts) • Chemical Abstracts (CAS) • Science Citation Index Expanded (ISI/Thomson Scientific) • Web of Science (ISI/Thomson Scientific)

BioPharm International (ISSN 1542-166X [print]; ISSN 1939-1862 [digital]) is published monthly by MultiMedia Healthcare LLC 325 W. First Street, STE 300 Duluth, MN 55802. Subscription rates: $76 for one year in the United States and Possessions; $103 for one year in Canada and Mexico; all other countries $146 for one year. Single copies (prepaid only): $8 in the United States; $10 all other countries. Back issues, if available: $21 in the United States, $26 all other countries. Add $6.75 per order for shipping and handling. Periodicals postage paid at Duluth, MN 55806, and additional mailing offices. Postmaster: Please send address changes to BioPharm International, PO Box 6128, Duluth, MN 55806-6128, USA. PUBLICATIONS MAIL AGREEMENT NO. 40612608. Return Undeliverable Canadian Addresses to: IMEX Global Solutions, P.O. Box 25542, London, ON N6C 6B2, CANADA. Canadian GST number: R12421333RT001. Printed in U.S.A.
A Biologics Partisan Divide

A bipartisan effort by the Senate Health, Education, Labor and Pensions (HELP) committee designed to lower healthcare and medication costs has led to a contentious debate between FDA and standards-setting organization, the US Pharmacopeial Convention (USP). The committee voted 20–3 on June 26, 2019 to advance legislation with proposals that include changes to patent policy, ending “surprises” in medical bills, increasing transparency in healthcare, and reducing roadblocks that delay generic drugs and biosimilars getting to market (1).

It is provisions in Section 207—designated as promoting biological drug innovation—that are proving controversial, pitting FDA and USP in an exchange of policy statements. According to HELP committee documents, Section 207 is intended to prevent delays in the licensure of biosimilar and interchangeable products by excluding biological products subject to regulation under the Public Health Service Act from requirements to follow United States Pharmacopeia compendial standards. A similar provision to make biologics exempt from meeting USP standards was removed from the final 21st Century Cures Act of 2016 (2).

“A biological product is so inherently complex and variable that the established structure of the USP monograph standards process does not serve it well, and in fact, can impede technological progress or innovation,” noted Steven Kiozolski, director of the Office of Biotechnology Products in FDA’s Office of Pharmaceutical Quality in an interview published by FDA (3).

Due to the inherent differences in biological products, the “sameness” standard used in USP monographs for chemical-based drugs cannot apply to biologic drugs, FDA argues.

USP, backed by patient groups and pharmacists, argues that the elimination of required standards would harm patients. In a letter to the committee, USP noted the role standards play in providing quality benchmarks; supply chain security; reliability and predictability for drug product development, manufacturing, and distribution; and promoting transparency and accountability that leads to patient trust (4).

“Essential to the framework that safeguards the quality and safety of medicines in the United States is the principle that public quality standards, required under the law, establish and articulate quality expectations for medicines, including biologics,” USP noted in the letter.

FDA has no legal authority to control the price of drugs. However, the agency notes it is “committed to facilitating increased competition in the market for prescription drugs through the approval of lower-cost, generic medicines” and “makes sure that safe and effective drugs are available to improve the health of consumers” (5). That’s a lot of priorities to balance in a high-stakes, highly partisan environment.

References

Rita Peters is the editorial director of BioPharm International.
As part of its ongoing support for the development and approval of competitive biotech therapies, FDA has updated and clarified standards and procedures for ensuring the quality and similarity of biosimilars. A new draft guidance recommends a process for developing comparative analytical assessment plans to demonstrate biosimilarity, with added details on procedures for conducting such assessments and for documenting quality in a range of production lots to make biosimilar development and production more predictable and efficient.

The new advisory replaces an earlier guidance on quality considerations for biosimilars issued in 2015 and updates a 2017 draft guidance on statistical approaches, both withdrawn later by FDA due to industry concerns and objections. This revised document presents strategies for conducting comparative analytical studies to assess biosimilarity to a reference product and for presenting scientific and technical information in the chemistry, manufacturing, and controls (CMC) section of biosimilar applications. There’s more detail on how comparative analytical assessments should cover key processes, including a product’s expression system, manufacturing process, physicochemical properties, functional activities, target binding, impurities, reference product and reference standards, finished drug product, and stability.

To address concerns about lot-to-lot variability, FDA advises biosimilar makers to include data from at least 10 reference product lots acquired over several years to fully assess reference product drift. Similarly, sponsors should assess 6–10 lots of the proposed biosimilar, including both investigational and commercial-scale lots, validation lots, and lots manufactured at difference scales. Additional sections clarify the submission of reference standards and data on functional activity to demonstrate any structural differences with a reference product. And FDA provides the usual caveat that it will consider alternative approaches from sponsors in analyzing and presenting requested data.

SUPPORT FOR INTERCHANGEABLES

FDA issued the revised quality assurance advisory soon after publication of a final guidance on developing interchangeable biosimilars, a much-anticipated and hotly debated option for manufacturers. That document updates a draft guidance from January 2017 that attracted voluminous comment from multiple stakeholders. The new version instructs manufacturers on conducting switching studies, which FDA says it usually will require to approve an interchangeable label. While some firms consider extensive switching studies unnecessary, FDA justifies this approach as important for demonstrating fully that safety and efficacy are not affected by changing treatment from brand to interchangeable, a finding that is considered important to gain the trust of physicians and patients in pharmacy-level switching.

Important for biosimilar makers, the new guidance supports the use of non-US-licensed comparator products to conduct tests and to produce the data needed to demonstrate interchangeability. Even though FDA wants data from bridging studies to foreign-made reference products, manufacturers still may benefit from leeway to obtain samples of biologics that often are less costly outside the United States and can help reduce the cost and time for developing interchangeable therapies. Sponsors developing interchangeable products may be eligible to...
gain a year of market exclusivity for being the first to develop such a copycat therapy.

The new guidance also appears less didactic by dropping the frequent use of terms such as “residual uncertainty” and “fingerprint-like” similarity between reference and interchangeable products, which were common in the earlier advisories. FDA here aims to set general standards and requirements for these therapies but notes, however, that the specific data and testing needed to document interchangeability may vary with the structural and functional complexity of the product and with clinical experience with the reference product. Sponsors may seek to justify an exemption from switching studies, and FDA says it will take a flexible approach.

**EYE ON INSULIN**

In moving forward on standards for interchangeable products, FDA sets the stage for the development and approval of competitive insulin products, which are scheduled to transition to biologic status in March 2020. There is great anticipation that interchangeable insulin therapies will provide important alternatives to current costly diabetes treatments as multiple sponsors move into this field. These issues were discussed at an FDA public meeting in May on the future of biosimilar insulins, just days after the appearance of the interchangeable biologics guidance. As a relatively simple biotech product that has been available as a drug for 100 years (but ineligible for generic competition), manufacturers expect to be able to utilize a rather streamlined and straightforward process for documenting similarity and interchangeability for these widely used therapies. Firms planning to produce competitive insulin therapies propose that FDA require data from only one, small immunogenicity study, as opposed to multiple switching studies, and that analytic characterization, bridging studies, and reliance on pharmacokinetic and pharmacodynamic data should provide ample support for interchangeable insulins.

FDA says it plans additional guidance on data needed on product container closure systems and delivery device constituent parts to support the presentation of a proposed interchangeable product. Of interest is how a biosimilar or interchangeable determination may be affected by delivery of insulin through a pump or over-the-counter device.

So far FDA has not approved any biosimilars as interchangeable, but this is expected to change with this added clarification of regulatory process and requirements. The rising price of insulin therapies has become one of the most contentious issues in the escalating drug pricing debate, with Congressional hearings generating multiple legislative proposals on this issue, many supporting the development of biosimilar and interchangeable products.

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**Federal Court Decides US Stem Cell Clinics Adulterated and Misbranded Products**

FDA announced on June 4, 2019 that a US District Judge in the Southern District of Florida held that US Stem Cell Clinic LLC, of Weston, Florida, and US Stem Cell Inc., of Sunrise, Florida, and their Chief Scientific Officer Kristin Comella, PhD had adulterated and misbranded a stem cell drug product made from a patient’s adipose tissue. The ruling came after the US Department of Justice initiated action in May 2018 to seek a permanent injunction against the clinics after attempts by FDA to work with the company to become compliant with regulations failed.

“In the case against US Stem Cell Clinic, the clinic and its leadership have put patients at serious risk through their disregard of the law and prior FDA warnings. This decision today is a victory for the FDA’s work to stop these bad actors and to protect patients,” said Peter Marks, MD, PhD, director of the FDA’s Center for Biologics Evaluation and Research, in a press release (1).

**Reference**


—The Editors of BioPharm International
Gene therapies are becoming a fast-growing area in the biopharmaceutical industry, creating a crowded pipeline of products in need of manufacturing. FDA expects the agency will receive more than 200 investigational new drug applications for cell and gene therapies by 2020, with the agency predicting that it will be approving 10–20 cell- and gene-therapy products each year by 2025 (1).

Because gene therapies use the patient’s own body to manufacture the active ingredient, the treatment is specific to that patient. This tailoring can often be a more effective and longer-lasting treatment, according to Chris Murphy, general manager for viral vector services at Thermo Fisher Scientific. “Because of these developments, novel, life-changing therapies are being realized that may transform the lives of patients in need.”

This growth is causing contract development and manufacturing organizations (CDMOs) to take a look at their portfolios as their clients require more and more biologics-related services. Catalent has been focused on the clinical pipeline of gene therapies and anticipating the need for commercial manufacturing. “There is an abundance of gene therapy product candidates in the pipeline, and on the heels of market approvals of gene therapy products in 2017 and the influx of venture capital funding for gene therapy companies, FDA approved a second gene therapy this year. We see this as a watershed moment, where gene therapies are gaining regulatory acceptance and setting a precedent for other programs in the pipeline,” says Bernie Clark, vice-president, marketing and strategy, Catalent Biologics & Specialty Drug Delivery.

According to Clark, outsourcing the manufacture of gene therapies requires relatively low volumes to fulfill demand, making it an attractive option. To better service its clients’ outsourcing needs in this area, Catalent acquired Paragon Bioservices, Inc., a viral vector development and manufacturing company for gene therapies with expertise in adeno-associated virus vectors, in May 2019 (2).

Clark believes the acquisition will help Catalent accelerate its growth. “Paragon offers its partners GMP-compliant manufacturing capacity, scale-up expertise, and customized downstream processing without the added time, costs, and risks of building a new viral facility,” says Clark. “The acquisition also brings complementary capabilities that will fundamentally enhance our biologics business and our end-to-end integrated biopharmaceutical solutions for customers. We have also gained the experienced leadership and dedicated team at Paragon, who will continue to run the business going forward, bringing their expertise and capabilities to the Catalent family.”

Thermo Fisher Scientific Inc. took a major step into the viral-vector manufacturing arena with the announced purchase of Brammer Bio, a leading viral vector CDMO, with 600 employees and locations in Massachusetts and Florida. The purchase agreement, announced in a March 24, 2019 press statement, is for approximately $1.7 billion in cash. Brammer Bio joins Patheon, acquired in 2017, and Fisher Clinical Services, which have been combined to form the Thermo Fisher’s Pharma Services business (3).

The acquisition enhances the company’s pharmaceutical and biotechnology customer value proposition by combining cell-culture media, single-use bioprocessing capabilities, analytical instruments, and related consumables. “Brammer Bio is an exciting addition to our Pharma Services business. By sharing our combined capabilities, expanding commercial scale and broadening deep customer relationships, we will strengthen our position as a

Gene Therapies Propel Outsourcing Investment

The increasing growth in the cell- and gene-therapy markets is inspiring CDMOs to expand their services in this emerging biologic drug arena.
trusted partner to our pharma and biotech customers. We will accelerate advancements in gene and cell therapy, meet the increasing demand of the customers we serve and bring life-changing medicines to patients in need,” says Murphy.

**VIRAL VECTOR CHALLENGES**

Viral vectors are crucial to gene therapy because they deliver the therapy into the patient. “Viruses are evolutionarily designed to enter mammalian cells and reproduce themselves. Gene therapy harnesses this feature to transport the genetic material—or ‘active ingredient’—to target tissue or cells. Viral vectors are engineered to be safe for humans and can target specific cells or tissues in our bodies to maximize the effect of the treatment,” says Murphy. Thermo Fisher Scientific’s acquisition of Brammer Bio’s viral vector process development and manufacturing leverages capabilities in the company’s biologics business.

Clark also emphasizes the importance of viral vectors. “During gene therapy biomanufacturing, cells package the therapeutic genetic material into viral vesicles and secrete the vesicles into the media to be purified, then undergo formulation development to be ultimately filled into a vial or syringe,” says Clark. According to Clark, however, few innovators have the capacity, expertise, or resources to manufacture viral vectors. Catalent’s acquisition of Paragon helps the company manufacture viral vectors for their clients. “There are several types of viral vectors that can be utilized for gene therapy. Adeno-associated virus (AAV) is the vector most commonly used today in clinical trials and approved gene therapies, due to its safety and tissue-specific targeting abilities, and Paragon is one of the leading development and manufacturing providers for AAV vectors,” says Clark.

**EXPANDING OTHER SERVICES**

In May 2019, Thermo Fisher Scientific announced that it is investing more than $50 million into its global bioproduction capabilities. The expansion will provide additional capacity for manufacturing single-use bioprocess container (BPC) systems. In Cramlington, United Kingdom, the company will expand assembly capacity and add BPC systems manufacturing. The proximity of these capabilities to customers in Europe will shorten lead times and improve overall global efficiency, according to the company (4).

“In the United States, the company will expand cleanroom space for BPC chamber and related assembly production processes at its site in Logan, UT, and further expand capacity at its site in Millersburg, PA. Construction is expected to be completed by the end of 2020. “The demand for our bioproduction products and services continues to outpace the market,” said Cory Stevenson, president of Thermo Fisher Scientific’s bioproduction business, in a company press release. “These investments will expand capabilities across our existing bioproduction network while we look to extend our footprint into new regions to meet increasing customer demand for our industry-leading single-use technologies.”

To further develop its biologics offerings, Catalent is launching OneBio Suite, a new offering for the integrated development, manufacturing, and clinical supply of biologic drugs. The Suite is designed to address challenges that arise when accelerating programs to clinic or market, while also reducing complexity and risks from projects. The OneBio Suite is built on Catalent’s track record in biologic drugs development, which includes more than 115 global clinical trials and 11 commercially marketed monoclonal antibodies using the company’s GPEx cell line development technology, and 20 approved products through fill/finish and commercial supply to global markets, the company reports. According to Clark, the company has multiple initiatives in the works to shorten timelines and increase efficiency (5).

“Time is often lost for sponsors on the path to clinic from contract negotiation, site inspections, hand-offs, and poor communication between multiple vendors,” commented Clark in a press statement announcing the service. “Through our new OneBio Suite, Catalent is uniquely positioned to provide an integrated offering that can accelerate biologic development potentially shaving weeks to months off standard timelines and allowing our customers to get to clinic and market faster.”

**REFERENCES**


Early Development Pipeline

**Source of Nucleic-Acid Asymmetry May Advance Gene Therapy**

New research from the Institute for Research in Biomedicine (IRB Barcelona) published in the journal CHEM offers insight into the source of asymmetry between nucleic acid hybrids. This development may prove to be an important contribution to improving gene therapies (1).

The study, done in collaboration with the Centre for Genomic Regulation and the Institute for Advanced Chemistry of Catalonia, has analyzed the source and biological consequences of the asymmetry that occurs in RNA–DNA hybrids when the relation between purine (adenine and guanine) and pyrimidine (thymine and cytosine or uracil) bases differs in RNA and DNA strands (2).

According to the researchers, the results of the study indicate that in contrast to the homoduplexes of DNA or RNA, RNA–DNA hybrids show intrinsic asymmetry, which suggests that this property is important for biological function and for biotechnological applications. When the DNA of the hybrids is rich in pyrimidine bases, the duplex is more stable and rigid than when the DNA chain is rich in purine bases. This symmetry, according to the researchers, may lead to improvements in the efficiency of therapies based on hybrids, such as antisense therapy, which can, for instance, control the regulation of genes that contribute to the progress of cancer progression and other diseases, as well as CRISPR-Cas9 gene-editing technology, which allows a target gene to be cut and edited.

“Thanks to a combination of theoretical and experimental methods, we have been able to understand the relationship between the sequence and stability of DNA–RNA hybrids—structures that form in the cell spontaneously and that have enormous therapeutic potential,” said Modesto Orozco, head of the Molecular Modeling and Bioinformatics Laboratory at IRB Barcelona and senior professor at the University of Barcelona, in an institute press release. “Our results will allow further development of much more efficient methods to block and edit genes and that can potentially become therapeutic alternatives for diseases for which there are no effective treatments.”

**References**


**Tiny DNA Reader to Advance Anticancer Drug Development**

Scientists from Osaka University have developed a solution to the challenge of studying anticancer drugs incorporated into single strands of DNA (1).

According to the university, despite constant effort from researchers to develop new and improved therapies to eliminate cancer cells—or at least halt their replication—a limited understanding of exactly how these drugs work can sometimes make it difficult to advance treatments. One such treatment, trifluridine, is an anticancer drug that gets incorporated into DNA as it replicates. While similar to thymine, one of the four nucleotides that make up DNA, trifluridine cannot bind to thymine’s partner nucleotide, adenine. This destabilizes the DNA molecule, resulting in aberrant gene expression and, ultimately, cell death. However, exactly where trifluridine gets incorporated into the DNA is unknown because it is not distinguished by traditional DNA sequencing methods, hampering efforts to fully understand and develop the technology.

In a study published in *Scientific Reports*, the university researchers developed a DNA sequencing method that could distinguish the drug molecules from normal nucleotides in short strands of DNA (2). Using microscopic probes, the team passed an electrical current across a distance approximately 65,000 times smaller than a grain of sand—a gap just wide enough to fit a strand of DNA.

“Using this single-molecule quantum sequencing method, we successfully identified individual molecules in the DNA based on differences in electrical conductance,” said lead author Takahito Ohshiro in a university press release. “For the first time, we were able to directly detect anticancer drug molecules incorporated in the DNA.”

The researchers report that the conductance of trifluridine was lower than that of the four native nucleotides, which also displayed divergent conductance values, allowing it to easily be distinguished in the DNA sequence. Based on these values, the team successfully sequenced single DNA strands of up to 21 nucleotides, pinpointing the exact insertion sites of trifluridine.

“Now that we have the ability to determine exactly where the drug is incorporated, we can develop a better understanding of the mechanism involved in DNA damage,” said senior author Masateru Taniguchi in the release. “We expect that this technology will aid in the rapid development of new and more effective anticancer drugs.”

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Catching Up Downstream

Although downstream efficiency still lags behind upstream, engineering-driven innovation is breaking through boundaries.

AGNES SHANLEY

The biopharmaceutical market is challenged by growth, restructuring, and time-to-market pressures. For contract development and manufacturing organizations (CDMOs), development timelines for standard vanilla antibodies have been cut in half over the past few years, says Uwe Gottschalk, chief scientific officer at Lonza. “That means going from DNA to clinical trials within one year,” he says, “and development times for all the individual steps entailed, from cell-line construction to a new manufacturing process, have all been shrinking dramatically.”

For downstream bioprocessing, the greatest test has been addressing ongoing productivity gains upstream. “There is a gap, and it is widening, between what is coming out of the fermenters and the capabilities of downstream processing systems,” says Gottschalk. While upstream processing is volume-driven, unit operations downstream are mass driven, he says. One can make a kilogram of antibodies in a 1000-L bioreactor, or increase the output to 10 kilograms by increasing expression levels and overall yield, says Gottschalk. But to handle the larger amount downstream, at any capacity, will require chromatography columns that are 10 times larger, or that are run in 10 cycles, he says.

Market pressures have driven improved process technologies, which some observers refer to as “next-gen” bioprocessing, a category that includes process intensification and the connection of different unit operations, as well as continuous processing, both up and downstream. “These improvements allow manufacturing to run more efficiently and produce higher yields in less time and space, reducing capital investment,” says Andrew Bulpin, head of process solutions at MilliporeSigma. “By 2020, we expect that approximately 20% of today’s molecular pipeline will be manufactured using elements of next-generation bioprocessing or continuous manufacturing,” he says.

Downstream, these improvements include combined unit operations, alternatives to chromatography, new process modeling techniques that allow for the visualization of “the golden batch,” and analytical approaches including greater use of process analytical technologies (PAT). While the gap with upstream may still be there, it is being addressed. This article highlights trends.

PROCESS INTENSIFICATION

Behind many of the new advances is process intensification, which improves facility productivity by focusing on terms of kilograms of product manufactured per year, per square meter of facility footprint, says Peter Levison, executive director of...
“Chromatography is a potential bottleneck downstream because sorbents have a finite binding capacity, but process intensification allows feed to be pre-concentrated prior to adsorption,” he says. “In addition, one can move from batch to continuous chromatography to maximize the adsorptive capacity of the sorbent,” he says, noting the benefits of single-pass tangential flow filtration (TFF). As higher concentration formulations become the rule, filtration systems will have to handle concentrated and more viscous solutions without damaging product, he says, and virus and sterile filters are being developed to address these challenges.

Bulpin also notes the importance of process intensification “Our work with customers has shown that facility throughput can increase by up to 75%, simply by converting one or two unit operations,” he says.

Alex Chatel, product manager at Univercells, singles out TFF systems as a major process-intensification-based advancement. TFF enables a large increase in concentrations in a single pass, so that feed streams needn’t be recycled many times over through the membrane, he says. Bulpin recalls one case in which a manufacturer installed a single-pass TFF device, which now provides inline feed stream concentration and/or dilution, eliminating the need for holding tanks and intermediate process steps. “With slight modifications to a couple of unit operations, companies can incorporate a more continuous approach while reducing their manufacturing footprint and scale,” he says.

Using process intensification, unit operations are being integrated so that, for example, cell removal and early clarification may be captured in one step, instead of the three or four that were previously required (i.e., typically centrifugation followed by depth filtration, sterile filtration and capturing column), says Gottschalk. As a result, some downstream processes have been reduced from 10 to three or four steps, and, in the future, he believes, two or three steps will be possible.

**TRIUMPHS OF ENGINEERING**

The move to continuous chromatography (i.e., having multiple columns running side by side) has also been a significant advance, says Chatel. Univercells, for example, is developing continuous-process-based platforms and is working on virus manufacturing platforms in projects sponsored by the Gates Foundation. “All are batch processes because they depend on binary loops and they need regeneration, but they are run so that when one is purifying product, the other column is being regenerated, resulting in a continuous stream of product output. Advances in engineering and mathematics enabled this to happen, and have drastically reduced the amount of resin required,” he says.

The result of all these improvements has given manufacturers access to a “scaleable toolbox” that can enable batch, continuous, or hybrid process development and scale up using stainless steel or single-use systems, says Levison. Examples he cites include, not only processes that enable continuous chromatography, but depth filters designed to clarify higher cell density fed batch culture; continuous diafiltration and continuous concentration; and the introduction of large-scale prepacked chromatography columns and new single-use chromatography skids to complement them. “With associated products such as sterile connectors, biocontainers, and single-use filters, a closed, fully connected downstream processing train can now be assembled, allowing for rapid scale-up and process implementation,” says Levison.

There has been a shift to single use downstream, Gottschalk says. In some cases, single-use systems can run at close to 100% variable cost eliminating the need to depreciate costs for the facility and stainless steel equipment, he says. Gottschalk sees increased use of convective, instead of throughput-limited chromatography media, as a key trend. “Filters are developed with the ligands that would normally go on chromatography resins, and used in a membrane, monolith, or depth filter,” he explains.

Although some research groups and companies, most notably Merck & Co., have been exploring end-to-end continuous biomanufacturing, more manufacturers are using continuous processing strategically, both up and downstream. Univercells has developed a continuous production system in which cells are grown in batch mode but different process steps are run continuously. “We need to get away from the old approach, in which you send all the contaminants downstream,” says Chatel. As Gottschalk says, continuous processing was always used upstream for sensitive molecules that could not endure conditions in the fermenter (e.g., enzymes such as Factor A for hemophilia treatments). Perfusion fermentation was essential in order to keep the molecule intact, but the approach has since been used with other more stable molecules such as antibodies, and is now moving into the broad biopharma world, he says.

Continuous processing is already a reality for single-use systems, Levison says. “We can currently clarify cell culture media continuously using depth filtration; purify product using continuous chromatography; carry out continuous virus inactivation; and then complete the ultrafiltration and diafiltration stages using continuous single-pass filtration technologies,” he says.

Trends for the future relate more to the modularity of the continuous downstream platform, with connectivity between each unit operation and integrated PAT and automation and control platforms, Levison says. For a truly continuous upstream process, a perfusion bioreactor could be used upstream, but it would require continuous cell culture fluid harvesting. Current cell-retention technologies based on hollow fiber filtration are prone to fouling, he says, so the composition of the harvest is variable and changes over time. One alternate
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could be to use acoustic wave separation (AWS), and platforms using AWS should be commercially available in the near future, he says.

MODELING AND PAT
The biggest change in bioprocess automation has been the introduction of the model-driven approach, says Bulpin, who explains that this approach allows users to define process, interactions, and contexts in form of parameterized data. This helps to reduce complexity, increase flexibility and reusability, and enable dynamic re-configuration of systems and processes. Model-driven design can lead to increased quality by increasing visibility, providing higher abstraction layers with less custom code, and empowering the domain experts by focusing on the process rather than underlying technology, Bulpin says. In the long term, he expects it will lower the sensitivity to change and help to bridge the gap between business and technical domains.

At the same time, analytical methods are improving, and use of PAT is increasing. Chatel has seen advances in sensors for pH, dissolved oxygen, and traditional measurements, that have allowed companies to adopt monitoring and control practices for bioreactors and other steps such as chromatography. “Future sensors will be able to detect metabolic concentrations in cell cultures (e.g., measure titers at line and provide feedback control over feeding strategies) to reduce burden of contaminants and ultimately lead to more lean processes and reduce processing time,” he predicts.

Noting the increased use of Raman spectroscopy-based methods, Gottschalk says, “We can now monitor the most relevant critical parameters online or at-line so that, after we have manufactured the batch, we know whether it is acceptable and can be approved or not. In biopharma, we have one or two critical assays that need to be run at the end of production, such as the adventitious virus test, which cannot currently be brought online, but most other analytical tests can be run online,” he says.

Bulpin sees artificial intelligence (AI), primarily machine learning, as driving future improvements in PAT. “This approach should significantly improve quality and process robustness; combined with near-real-time data acquisition, it will enable closed loop for the process execution,” he says, improving communications and interaction between enterprise systems and manufacturing field I/O (inputs/outputs), including sensors, actuators, analysers and drives.

As more bioprocessing steps are automated, the approach is also proving important for sampling, says Gottschalk. Lonza is working on data mining and new approaches using machine learning and other artificial intelligence concepts. “The goal is to use advanced concepts and multivariate data analysis to ensure that we run only the ‘golden batches,’” he says.

Over the next few years, Levison expects new inline or at-line tools to become available to monitor both critical quality attributes and critical process parameters. “Whether this will involve new sensing and detection technologies or multi-attribute measurements (MAM) based on existing technologies, or indeed a combination of both approaches, remains to be determined. What is clear is that as we move towards advanced analytics and PAT, more data will be generated more frequently so the demands on the data storage, management and handling will increase and this all needs to interact with the process control system. AI and the Internet of Things will play a big part in these advances, which all fall under the umbrella of Industry 4.0,” he says.

One of the benefits of Industry 4.0 would be ability to use simulation (e.g., in the digital twin approach). “Provided that the digital twin can be shown to be a true twin of the real process, then its use in method development and optimization may become a reality (e.g., for determining process limits without carrying out a detailed design of experiment). If the digital twin could predict batch-to-batch variability and process robustness then perhaps this could reduce time to market of new medicines directly impacting on patient health,” says Levison.

BUFFER MANAGEMENT
One practical area where downstream efficiencies are being improved is in buffer and waste management. “Single-use technologies offer benefits because they reduce cleaning and cleaning validation requirements,” says Levison. “With the introduction of single-use mixers and single-use biocontainers, we have opportunities for storage of buffer concentrates with in-line dilution and/or conditioning to generate the buffer on demand at the point of use,” he says. Fluid waste can be collected in biocontainers which can then be aseptically disconnected and disposed of, he adds. However, as facilities become smaller, buffers continue to pose complex storage and logistical challenges and can become a bottleneck for single-use production, Levison says. One solution to increase facility buffer capacity is through the use of concentrates or stock solutions, both enabled by single-use mixers and single-use biocontainers. Managing buffers this way and delaying the dilution of these concentrated solutions up to the point of use, allows single-use facilities to increase their buffer capacity and increase their productivity.

In the area of buffer management and maintenance, MilliporeSigma recently launched the BioContinuum buffer delivery platform, Bulpin says, which uses buffer concentrates and in-line dilution to deliver buffer directly into the system. The company expects the platform to permit an 18% reduction in cleanroom area requirements in a 2,000-L bioreactor facility; reduction in buffer costs by up to 16%; and more than 50% reduction in labor and capital costs for a facility that uses five 2,000-L bioreactors, he says.
Applying QbD to Upstream Processing

Using a QbD approach from early-stage development through commercialization can ensure that upstream processes are efficient and reliable.

SUSAN HAIGNEY

The concept of quality by design (QbD) has been part of the biopharmaceutical industry for more than a decade. While similar approaches to QbD are used in the development and manufacturing of large-molecule and small-molecule drugs, the complex nature of large-molecule drugs creates more factors that may affect process performances and the critical quality attributes (CQAs) of the drug.

Because biomanufacturing involves living cells, there are more reactions happening in a bioreactor than usually happen in chemical reactions, according to Bill Whitford, strategic solutions leader, bioprocess, GE Healthcare. “While we often understand nearly everything about a small-molecule reactor development pathway, we have only a partial understanding of all the interacting salvage, degradation, and biosynthetic pathways of mammalian cells. This includes even many relevant metabolic pathways, inductions, flux, rates, and turnover. Finally, we often develop and control bioprocesses using very surrogate key quality indicator (KQI) and critical process parameters (CPPs). Rather than measuring precisely what we know (or suppose) to be relevant, we monitor such general parameters as pH, oxygen, trypan-blue exclusion, and such token or generally representative metabolites as glucose or lactate,” says Whitford.

According to the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ Consortium), a collective of more than 35 biopharmaceutical companies, there are also differences between the use of QbD for large molecules versus small molecules when considering antibody-drug conjugates (ADCs). ADCs are complex molecules composed of an antibody linked to a cytotoxic small-molecule drug. As noted by the IQ Consortium, companies that develop ADCs state that CPPs and CQAs may also differ between the drug-linker (DL) component of the ADC and the related small-molecule drug because the DL represents a small portion of the total mass of the ADC. Unlike small-molecule drugs, the dosing frequency and scheme of the ADCs also may dilute the impurities in DL intermediates.

Another difference between small-molecule and large-molecule drugs, according to the IQ Consortium, is that in early development, a platform process approach may be applied, which may accelerate timelines and reduce development burden.
**QbD IN UPSTREAM PROCESSING**

QbD is used in cell-culture expansion, cell harvesting, and cell-culture media development. However, according to the IQ Consortium, many companies find that clone selection and the development of the production-stage bioreactor process benefit most from the application of QbD due to the following: “In clone selection, the final producer cell line is defined, which is then used for the commercial process and throughout the lifecycle of a product, while the N-stage production bioreactor is considered to be the most critical step with respect to the CQAs and essentially determines the final product quality from an upstream perspective. In principle, all upstream process steps can benefit from a QbD approach and examples where companies apply those also depend on the assessment of (business) risks,” a representative of the IQ Consortium says. Predefining development goals for titer ranges and product quality profiles, as well as assessing risk, also benefit from QbD.

The development of robust processes that include good cell culture performance can be done through design of experiments (DoE). In early stage development, QbD can be used to identify a quality target product profile (QTPP), according to the IQ Consortium. Late-stage applications include process characterization studies to decide on one-factor-at-a-time (OFAT) experiments versus DoE-based experiments and the defining and classifying of parameter ranges. “Currently, a claim of a design space is not pursued by most companies. However, achieving in-depth understanding is a goal (e.g., by advanced data analysis approaches). Also, detailed risk assessments can play an important role in late-stage activities. Commonly, the knowledge gained in process characterization studies (PCS) will be included in the control strategy,” the IQ Consortium states.

Performing small-scale DoE by applying verified scale factors and using a risk-based approach are key to implementing QbD in upstream processing, says Whitford. “The final product will be a verified design space, clearly communicated—being mindful of using accepted terms and definitions—to regulators in the submission. Currently, upstream process development focuses on digitally transformed and intensified biomanufacturing,” Whitford states.

**Bioreactor process development has been positively impacted by the use of QbD and empowered by process analytical technology.**

Bioreactor process development has been positively impacted by the use of QbD and empowered by process analytical technology (PAT), according to Whitford, by enabling continuous monitoring of process parameters and product characteristics throughout development and manufacturing. “In the pursuit of process intensification, the influence of altering process parameters on product characteristics can be rapidly assessed. This contributes to increasing an understanding of what types and ranges of bioreactor parameters provide acceptable product quality. This mapping of the relationship between bioreactor process characteristics and product CQAs will define a bioreactor design space, an important element in both ensuring a robust process as well as current FDA expectations in filings,” says Whitford.

**USING QbD TO DEVELOP CELL CULTURE PROCESSES**

Because cell culture has a direct impact on production efficiency and product quality, using DoE can help determine the relationship between cell-culture process parameters and product quality, which then helps determine the design of the operation space for a process. DoE can also help determine the needed sensor technologies to establish robust closed-loop model predictive controls, according to Whitford. “Unlike many other production processes, biomanufacturing has classically been hindered by a lack of real-time product attribute measurement. Even in nominally identical bioprocesses, we can see diversity in product quality due to variability in (e.g., such raw materials as cell culture media). Using a QbD/PAT strategy directs the implementation of advanced near-real time monitoring through the application of newer, automated sampling techniques and multivariate data analysis. This supports advanced in silico modeling that can not only help control the process, but predict product quantity and quality outcomes,” says Whitford.

Implementing a feedback quality control strategy, as opposed to a recipe-based control strategy, when using QbD in developing cell culture processes may be the best approach, says Brandon Downey, principal engineer, R&D at Lonza, because of the challenges cell-culture processes present. “Many [cell culture] processes have successfully used a recipe-based control strategy, determined using a design space approach, to manufacture quality product. Nevertheless, cell culture processes pose some unique challenges when attempting to employ a recipe-based control strategy,” says Downey. And, the large number of raw materials used in cell culture can make understanding the variation of each component difficult. “Although fundamental understanding of how
variations in common raw material attributes impact a cell culture process is increasing, this knowledge is still far from complete enough to encompass the variation that has been observed in the cell culture media,” he states.

Using a QbD approach to define objectives for establishing a new manufacturing facility may be beneficial.

The behavior of the cell culture process may also be impacted by variations in unmeasured impurities in raw materials, says Downey, making it difficult to determine the total impact of raw material variation on the cell culture process. “Finally, the cells in the production cell culture step uniquely determine many of the structural attributes of biologic molecules which could (and in some cases have been clinically shown to) influence the function of the molecule.”

QbD in Cell Line Selection

When it comes to cell line selection, QbD can be used in the selection of a final clone during early-stage development by using prior knowledge to predetermine clone selection criteria including cell-line stability, culture performance, and product quality attributes. “As the selection process progresses, more clone-specific knowledge is gained, and the process continues until the clone that best fulfills the selection criteria is identified,” according to the IQ Consortium.

Whitford stresses that even though many bioprocessing platforms have been around for decades, individual process optimization is beneficial to individual cell strains, recombination techniques, cloning approaches, and culture systems. Continuous monitoring is being enabled by PAT-derived systems, says Whitford. In addition, highly accelerated bioprocess development is supported by new fully-automated, single-use multi-parallel bioreactors. “Employing some of the process monitoring and design approaches … in the context of high throughput and automated systems for process development is now very popular. For example, some vendors offer as many as 24 fully featured, single-use, 250 mL mini-bioreactors supporting process development and optimization as well as selection or development in scale-down studies,” says Whitford.

Medium selection, however, varies based on practices and needs, according to the IQ Consortium. “For example, companies that employ a platform process approach, including a platform cell culture media system, typically do not require media selection for molecules that fit the platform process. However, for atypical modalities that do not fit the predefined selection criteria, media screening can be done to select the condition which yields attributes that best fit the criteria,” says the IQ Consortium.

QbD in Equipment Selection

Using a QbD approach to define objectives for establishing a new manufacturing facility may be beneficial. Risk assessments can be used to evaluate different equipment against the predefined objectives. The knowledge gained through this process can show how different equipment may impact the objectives, according to the IQ Consortium.

QbD can also be applied to the evaluation of equipment performance.
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The suitability of equipment can be established through experimental designs, says Whitford. “This is generally accomplished through either limited experimentation at scale, or through studies in scale-down models, employing prior understanding of the scale-factors involved in extrapolating results to full-scale manufacturing. Premier single-use system vendors have for years been supplying much information to aid in such studies. One example here is CFD data on single-use mixers and various scales, fill volumes, impeller speeds, and viscosity of diluents. We have seen such techniques applied to the selection of single-use bioreactors and mixers, perfusion apparatus and even dynamically feed-back controlled inline buffer conditioning systems.”

When it comes to late-stage development, the IQ Consortium agrees that small-scale models (SSMs) are essential to QbD when scale-down models must be qualified to show they represent large-scale operations. “SSMs are used in PCS to understand the process and determine parameter ranges and classifications. Given that the PCS experiments are often large and complex, it is important to balance between representativeness and throughput of the SSMs,” according to the IQ Consortium.

CHALLENGES IN QbD IN EARLY DEVELOPMENT
Designing quality into the biopharmaceutical process early on can be difficult because the nature of the protein may not be fully understood, says Whitford. There is also a range of variants, such as glycosylation in monoclonal antibodies (mAbs), that occurs when biologics are generated, which can affect both biological activity and/or the secondary or tertiary structure of the molecule. “This can influence not only the entity’s activity but its stability in storage,” says Whitford. “Secondly, we don’t yet fully understand the conditions of cell culture that will control those molecular properties. Often, they are discovered more empirically than by pathway and structure understanding. Finally, we are only beginning to implement comprehensive monitoring of the bioreactor process, metabolites, and products that can provide robust specific, relevant, and predictive process parameter values and product quality assessment,” Whitford adds.

A robust cell culture platform process can ensure that CQA profiles are consistent from the early stages of development through commercialization.

A robust cell culture platform process can ensure that CQA profiles are consistent from the early stages of development through commercialization, according to the IQ Consortium. They also warn that using in-licensing molecules may present unanticipated challenges. “This might, depending on the development stage of the in-licensed molecule, shift focus from ‘design’ to comparability.”

CONCLUSION
The implementation of QbD into the bio/pharmaceutical industry aligns with regulatory and industry efforts to ensure the efficacy, safety, and quality of medications. The complex nature of biologics adds additional complexity to developing and manufacturing these innovative therapies. Using a QbD approach from early-stage development through commercialization can ensure that upstream processes are efficient and reliable.

“As biologics increasingly take the form of novel modalities (e.g., not mAbs), it will become increasingly important to be able to control the quality of biologics so that the structure—functional relationships of these new classes of compounds can be better understood in the clinic. Furthermore, as medicines become more personalized, the ability to tailor the structural attributes of biologics molecules that are dictated by the process will become increasingly important. QbD will likely continue to play a foundational role in creating the processes which can robustly deliver these compounds,” concludes Downey.
Manufacturing

Design Considerations for a Commercial Cell and Gene Therapy Facility

The commercialization of cell and gene therapies has become a reality, prompting deeper considerations of logistics, technology, and design for manufacturing facilities.

FELIZA MIRASOL

With the recent FDA approvals and commercialization of cell and gene therapies in the US market and a pipeline of cell and gene therapies progressing toward regulatory review, there is increased focus on establishing commercial manufacturing facilities for these complex biotherapeutics. The workflows of a cell therapy or a gene therapy differ from each other and from those of therapeutic antibodies, so the design and layout of the manufacturing facility must take these workflows into consideration.

WHAT TO CONSIDER

“There are multiple considerations to take into account, perhaps most important, though, are capacity utilization at full scale and space efficiency,” says Phil Vanek, general manager, Cell and Gene Strategy, GE Healthcare. “This means several things, including how the materials and personnel will flow throughout the facility (unidirectional) and how unit operations (individual processing steps) will be combined to build out a complete process.”

Further, the considerations for cell-therapy versus viral-vector manufacturing (for in-vivo gene therapy or as a starting material for ex-vivo gene therapy) can be quite different from each other, adds Jonathan Fortin, global director of Engineering, Lonza. “Viral-vector manufacturing lends itself to a pre-defined manufacturing process flow and equipment design, which leads to greater uniformity in scale-dependent footprint for multiple processes. Cell therapies, on the other hand, often have unique processes that limit the level of flexibility possible in suite and general equipment configurations,” Fortin explains.

He emphasizes that it is necessary to conduct a keen assessment of shared processing areas, taking into account multi-product segregation needs. For example, closed processing steps (e.g., pre-transfection bioreactor stages for viral vector) and lower utilization activities (e.g., final filling followed by product

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changeover or media preparation) could use shared spaces, driving up efficiency. In addition, material, personnel, and waste flows require special attention for the current design and in support of future expansion needs.

“As a contract development and manufacturing organization, building facilities to accommodate for multiple current and future customers—and, therefore, multiple known and unknown products, processes, and setups—and having a flexible floor plan are critical. We need to be able to accommodate for multiple products and different modalities in different phases, each with the ability to scale up operations to commercial-scale manufacturing in one facility,” Fortin states.

“For product-specific setup areas within the facility, such as dedicated suites, the key aspects come down to the nature and the stage of the product: autologous versus allogeneic, clinical going towards commercial versus commercial. We try to optimize the capital cost for our customers and for us by maximizing the throughput and flexibility of the facility,” he adds.

In addition to current processing needs, Vanek notes that a facility design must be flexible enough to accommodate future changes to any processes. “Tomorrow’s processes may look very different from today’s, so anticipating what will be needed in terms of gases, power, and bench space should be contemplated. And then, finally, a digital solution encompassing electronic batch records, process monitoring, scheduling, and other key process attributes should be designed-in early to add efficiency and control to the process,” he states.

DIFFERENT FROM mABs
Considering the differences between monoclonal antibody (mAb) production and cell/gene therapy production is also important when establishing a cell/gene therapy facility. Already well-established, mAb production is typically batch-oriented (in this way, making it similar to allogeneic cell therapy production) and characterized by larger single-use bioreactor skids with large downstream chromatography platforms, often segregated into two or more rooms. In mAb production, Chinese hamster ovary cells are the workhorse of the industry, and the process is mostly, if not completely, closed, notes Vanek.

Cell and gene therapies, on the other hand, cover a large number of cell types, are often patient-specific (autologous), and are dependent on equipment made for other industries (e.g., blood processing or protein bioprocessing). Next-generation technologies have to consider the cell types and the batch approach (single per patient or scaled up). Common to each, however, would be design criteria to minimize risk in manufacturing, including unidirectional flow of personnel and materials in the cleanrooms, proximity to a quality control lab for in-process testing, and wholly integrated quality management/electronic batch records to reduce operations errors, states Vanek.

“We have to split this discussion into two parts for a successful comparison: first, viral vectors for gene therapy and second, cell and ex-vivo gene therapy,” remarks Senthil Ramaswamy, head of R&D, Cell & Gene technologies, Lonza. Viral-vector production, he notes, has a number of parallels with mAb manufacturing, especially if using a suspension cell-culture process or an adherent format that has the footprint of a bioreactor rather than dozens of small multilayer cell-culture vessels in incubators. The needed infrastructure is also similar to mAb manufacturing, taking into consideration the unique segregation needs for viral vectors when multiple products are being manufactured in the same facility. “The scales of processing at the moment top out at about 2000 L of cell culture, which is in line with smaller mAb manufacturing processes,” Ramaswamy states.

On the other hand, cell and ex-vivo gene therapy currently have different facility needs for two primary reasons: need for scale-out rather than scale-up in the case of autologous therapies and the highly manual, and often open, nature of processing requiring aseptic processing operations, Ramaswamy explains. “Facilities are commonly grade B cleanrooms with grade A biosafety cabinets (BSC) and incubator banks. These requirements will see a dramatic change as closed automated operations (e.g., Lonza Cocoon technology for autologous or small-scale cell and gene therapy and suspension single-use bioreactors to scale up larger-scale cell therapies) are adopted for these products.”

Another important difference is the high level of tracking and tracing, which is crucial in cell and gene therapies, especially autologous patient therapies, but not relevant to campaign-based batch mAb production. In addition, given that autologous cell therapy products often lead to a single dose for patients with limited treatment options and time, redundancy in manufacturing capability to proactively mitigate against any production disruptions must also be considered. “This is a stark difference from mAb or allogeneic products where a stock-up strategy can offer sufficient mitigation. Necessary, geographical proximity to patients can also drive multiple simultaneous manufacturing sites,” says Ramaswamy.

“A common factor between viral vector and mAb production is that many of the processing steps are/can be standardized and scaled. This makes the design of a commercial facility for such products relatively straightforward, even considering that there is no standard platform yet for viral vectors,” adds Bertus Markies, head of Value Chain Management, Lonza. “As stated by Senthil, the situation is radically different for cell therapy products. For both allogeneic and autologous products, the design of the facility heavily depends on the process. The technology in these fields is still far from mature, and a wide variety is seen
in what is used, ranging from differences in equipment, media, and cell-expansion to even small parts such as tubing sets. In essence, these technologies have high potential once they become industrialized.”

**EQUIPMENT/TECHNOLOGY**

Another important factor to consider for a cell and/or gene therapy facility is the equipment and setup. For mAb bioprocessing and viral-vector manufacturing, the equipment needed is similar, such as single-use bioreactors and standard harvest and downstream systems for clarification, tangential flow filtration, chromatography, and sterile filtration, according to Ramaswamy.

Both mAb bioprocessing and viral-vector manufacturing are usually divided into upstream and downstream processes. Upstream focuses on maximizing both the number of cells per bioreactor unit volume (e.g., cells per liter) and then the productivity of the cell upon induction of protein (mAb) or viral particle expression. “Getting these conditions just right requires balancing the cell line, expression system, media and growth factors, and bioreactor conditions—such as stir rate and gas transfer. For biologics and viral vectors, the cells are a byproduct of production and, thus, often get lysed and discarded in the downstream process, which is designed to optimize both the yield and the purity of the final product,” Vanek explains.

In comparison, cell therapies are different principally because the cell is not a byproduct, but the final product. “Therefore, production strategy is all about preserving the cell phenotype across multiple production steps and scales, meaning that keeping the therapeutically active cell is paramount, as well as keeping the cells from becoming contaminated by microbes,” states Vanek.

“For cell therapy today, it is often a mix of standard and process-specific equipment. Standard equipment would be BSC, incubators, centrifuges, vial or bag-filling systems, and controlled-rate freezing and storage capabilities (see Figure 1). Process-specific equipment are typically cell isolation, selection, and transfection systems,” Ramaswamy clarifies.

Single-use systems are technologies that are widely used in the bioprocessing and blood processing industries to protect product from contamination, and these systems are also appropriately sized for most cell and gene therapies, says Vanek. Single-use bioreactors that are either flask based or rocking based are widely used, for example, with stirred tank bioreactors being used in certain applications.

Cell collection and washing is mostly by centrifugation (either closed-system centrifugation or a special approach based on elutriation), states Vanek. “Simple filtration is not commonly used; new platforms are popping up claiming to be gentler or simpler, but none have yet been widely adopted,” he adds. “Scalability is key, and few platforms if any can move easily between small volumes (10s of mLs) to large volumes (100s of liters). What’s more, analytical platforms for in-process control and cell characterization are lacking in the cell therapy industry but are being actively pursued.”

It is important to note, however, where customization of single-use technology may be required. “Reliable sourcing and quality standards are key considerations when selecting single-use systems for cell and gene therapy because they are often not designed with these therapies in mind,” points out Ramaswamy. For example, for cell therapy, the cells are the final product received by the patient and are highly dependent upon the process and consumables used. Cell therapy production does not have the numerous purification steps and analytical tests that are standard to mAb production.

To be able to scale up viral vector manufacturing, bioreactors—with 3D spacing rather than a cell-stack setup—are needed with automated downstream processing and product fill. The use of bioreactors would also be needed for allogeneic cell therapy production as well as maximizing automation at commercial scale, according to Ramaswamy. “As we see more products entering commercialization, there is an urgent need to close and automate these processing steps leading to industrialization of cell therapy processes. This will lead to greater standardization in the future,” he adds.
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Residual Impurities

Analysis of Residual Impurities in Continuous Manufacturing

Real-time monitoring of product- and process-related impurities remains a challenge.

Cynthia A. Challerer

Continuous bioprocessing provides numerous benefits, from high product quality and consistency to smaller physical footprints, increased flexibility, and potentially lower capital and operating expenses. It also provides the greatest benefits if real-time monitoring of process parameters and product quality, including detection and quantitation of residual impurities, is performed. While advances in analytical methods and automation are occurring, practical implementation of real-time monitoring of product- and process-related impurities has yet to be achieved.

Ideal Solutions?

“In an ideal world,” asserts Byron Kneller, senior director for analytical and formulation development with AGC Biologics, “we would have simple, rapid, physicochemical tests for residual impurities.”

Unfortunately, there are different types of residual impurities. They can be classified as product- and process-related, and as chemical impurities (typically small-molecule process additives such as isopropyl β-D-1-thiogalactopyranoside, antifoams, antibiotics, etc.) or biological impurities from the host cell or processing steps (host-cell proteins [HCP], host-cell DNA, residual Protein-A, etc.).

“Technologies that utilize mass spectrometry (MS) capabilities are, at this point, the seemingly best choice for residual impurity detection, characterization, and/or quantitation when coupled with liquid chromatography (LC) systems,” observes Amit Katiyar, director of analytical and formulation sciences for Thermo Fisher Scientific. Multi-attribute methods (MAM) capable of detecting, quantifying, and characterizing multiple product quality attributes are currently being developed.

A triple-quadrupole system would be best suited for quantitative analysis of small molecules/peptides, with quadrupole time-of-flight systems more appropriate for the characterization and semi-quantitative analysis of larger molecules, according to Charles Heise, senior staff scientist for bioprocess strategy and development at Fujifilm Diosynth Biotechnologies.

Cynthia A. Challerer, PhD, is a contributing editor to BioPharm International.
An amalgamation of both technologies for a single quantitative measurement covering a wide mass range would be an ultimate solution, he adds. A series of standards could be used to generate standard curves for target impurities and the software needed for identification and quantification of peaks. Aspects of high-throughput proteomics analysis may also be applicable to the continuous manufacturing environment.

Other analytical techniques Heise highlights include in-line spectroscopic measurements such as Raman, Fourier-transform infrared (FTIR), ultraviolet/visible (UV/Vis), and refractive index methods that can give quantitative as well as qualitative, real-time analysis. “The particular technology used will depend on the impurity, its expected concentration range, and whether monitoring is performed during steady-state conditions,” he observes. New methods or advances in current technologies may also become available that allow in-line measurements using nuclear magnetic resonance (NMR) imaging, sensor arrays, or LC systems.

The final option, Heise says, is to do no process monitoring and rely solely on the quality-by-design data generated during process development to define the operating ranges and edges of failure.

PRACTICAL REALITIES

“In practice, the choice of method may be constrained by the availability of the instrumentation, turn-around time, analyst training, and potential interference from the complex matrices that may be involved in the downstream purification,” comments Ian Parsons, director of analytical development for biologics at Charles River Laboratories. His company has focused primarily on the use of high-performance LC (HPLC) and the enzyme-linked immunosorbent assay (ELISA), with MS and gas chromatography (GC) also used frequently.

“The pharmaceutical industry is still heavily utilizing traditional separation techniques such as size-exclusion, ion-exchange, and reversed-phase chromatography in addition to immunoassays such as ELISA and electrophoresis as the primary means for quantifying product- and process-related impurities,” notes Katiyar.

Often tests for process-related impurities require specialized reagents such as anti-HCP antibodies or natural products like limulus amebocyte lysate and can be more complex, adds Kneller. In addition, many impurities are heterogeneous (e.g., HCP) and thus not amenable to detection and quantitation using simple tests.

In practice for continuous processes, in-line confounded (e.g., spectroscopy) or secondary parameter (e.g., off-gas, pH, basic physical property) measurements are most user friendly, but accuracy of

In practice, the approach used to detect process impurities may be determined by available instruments, assays, time, and staff capabilities.

residual impurity detection is sacrificed for real-time control, as a result of the heterogeneous nature of the signal, or to allow for detection of a secondary response, according to Heise.

He also notes that off-line LC–MS, where mass windows can be limited by buffer solution and scanning rates, or LC–evaporative light scattering detection (ELSD), is typically used for small molecules, but these are not sensitive enough and can take too long to run for large-molecule manufacture.

These approaches could potentially be substituted for sensor technologies with equivalent modalities, according to Heise, such as the Octet system from Fortebio, which relies on biolayer interferometry. Similarly, he says that ELISAs for sensitive quantitation of biological impurities that have slow response times (material quarantining needed) could also be replaced with equivalent ‘dip-and-read’ methods.

The new technologies (e.g., MAM, MS for HCP analysis, automation for DNA, HCP, and Protein A residuals) are just starting to be introduced in the process development space, according to Katiyar. “The transition from exploratory to fully validated methods will be slower for such methods in a cGMP environment where method performance, equipment validation, and data-integrity measures need to be at the highest level. There will also be a significant cost aspect of not only reconfiguring current laboratories to accommodate the equipment, but also for training and/or hiring new personnel with expertise in these new technologies,” he says.

SENSITIVITY AND MATRIX CHALLENGES

At the process level, identifying the residual impurities present and the appropriate analytical assays, with the required detection limits and sensitivity ranges, to monitor them across the process is critical, stresses Dan Pettit, senior staff scientist for analytical development at Fujifilm Diosynth Biotechnologies. At the operations level, he notes that identifying suitably equipped labs to develop and validate methods for GMP analysis is vital because of the heterogeneity of process impurities and the consequent analytical issues they pose.

Heterogeneous process-related impurities, specifically HCPs, are often quantified in early-phase projects using generic kits that may result in key impurities going undetected during purification process development. “These impurities have the potential to cause problems at production scale,” Kneller says. Current ELISA techniques lack the ability to identify specific immunogenic proteins, thereby making the process
development aspect less strategic in design, agrees Michael Farris, scientific manager of analytical and formulation sciences with Thermo Fisher Scientific. “Building toxicology and immunogenicity databases for various HCPs and other process impurities would help fine-tune process development and, in itself, provide a deeper insight into process performance and robustness,” he asserts.

Identifying/developing suitably sensitive methods is also challenging, according to Pettit. “Typically, ppm or ppb levels of analyte in a complex mixture must be detected, and therefore, isolation/derivatization of the analyte may be required. These activities tend to be prohibitively expensive to outsource,” he observes.

In addition, isolation of impurities prior to quantitation may introduce artifacts or cause the generation of various altered states during the isolation process. For example, physical manipulation of samples prior to the detection of multiple analytes may alter the HCP antigen profile and relative abundance of the analytes themselves, according to Farris. Additional studies to understand the stability of isolated product(s) may therefore also be needed.

Other process impurities such as Long R3 IGF-1, which is a component of some cell-culture processes, have a tendency to adhere to certain plastics, thereby making accurate quantitation more difficult, Farris adds. Sampling instructions must be clearly defined so as to not artificially deplete the analyte during sampling and/or storage prior to analysis.

Parsons agrees that the main challenges are focused around developing methods of sufficient sensitivity that also minimize product and matrix-related interference, and thereby accomplish sufficient recovery of the analytes. In numerous instances, Farris points out that the quantitation and/or characterization of impurities is hindered by the API and/or the matrix composition.

“Immunoassays, such as ELISA, used for quantitation of host-cell proteins and leachables like Protein A are sensitive to extreme pH, high salt concentrations, and certain detergents. The typical means for overcoming the signal suppression or interference associated with their presence is to dilute the sample, which acts to decrease the sensitivity of the method to maintain acceptable precision and accuracy,” Farris explains. As a consequence, sensitivity constraints imposed by matrix/API interference must be a point of consideration when demonstrating that a method is fit for use for process characterization and/or process validation activities.

For products with small total batch volumes, the quantities of material required for analytical method development and sample analysis can also present a challenge, Parsons notes. “If method sensitivity is also an issue, an approach can be taken to spike an aliquot of upstream material with a higher known concentration of analyte and then demonstrate fold-removal of the analyte across the downstream purification step(s),” he comments.

**CONSTRAINTS IN CONTINUOUS BIOPROCESSING**

For continuous processes, the turnaround time of typical assays is a key issue, according to Kneller. “The challenge is to increase our testing throughput, decrease our reliance on heterogeneous reagents, and to find ways to either simplify the method types used or engineer robust, easy-to-use systems for more complicated analyses (e.g., mass spectrometry),” he says.

Real-time monitoring of representative material for modeling the residence time distribution in continuous processes is also required for process control, according to Pettit. “The range of appropriate analytical sensors for continuous monitoring of product specific critical quality attributes and impurities is limited. Additionally, sensor drift over the operating time, any on-going sensor calibration needs, and system suitability testing for quality critical attribute monitoring during continuous operation must be resolved,” he adds. “Here again, sensor technology could be the way to go (e.g., online Octet dip-and-read-type systems); however, the technology is not sufficiently developed yet. The final technology offerings need to be diverse, robust, accurate, reliable, and rapid,” he concludes.

For off-line analysis of residuals from a continuous process, Parsons notes that methods for aliquot/sample withdrawal are needed. In addition, the sampling frequency should be sufficient to provide a statistical sampling of the material flowing through the process in order to account for any transient variation that may occur while avoiding significant depletion of the process flow.

The analytical testing strategy associated with continuous processes can, however, be more demanding in terms of the number of sampling time points if in-line or on-line monitoring is not possible, according to Farris. “Process analytical technologies have generally been geared more toward product quality, cell-culture viability, and growth than active monitoring of process impurities. If a deeper understanding of any particular impurity is required, more complicated assays will likely be required,” he observes.

**RESIDUALS ANALYSIS WHEN MOVING FROM BATCH TO CONTINUOUS**

When moving from batch to continuous processing, it is likely that the same analytical methods for residual impurities can be used, but potentially in higher throughput versions. Offline analysis using LC, GC, ELISA, and MS should be possible as long as an appropriate sampling approach can be implemented, Parsons observes.

The throughput capability and time-to-result for the analytical method employed are also important aspects if at some point the impurity is deemed process critical, according to Darshini Shah, senior scientist and group lead for downstream process development at Thermo Fisher Scientific.
Thermo Fisher Scientific. Automation of methods (e.g., liquid-handling or use of systems such as the Octet for HCP analysis) can provide higher throughput both in terms of overall speed and analyst effort, according to Kneller.

The analytical target profile must also be evaluated to ensure methods are capable of monitoring fluctuating analyte levels throughout continuous processing. “Retention rates or biomass removal may result in periods of correspondingly lower impurity levels, and the analytical method must be sensitive enough and support a large enough dynamic range to be able to actively monitor the process range without the need to constantly repeat analyses to better target the operating range of the assay,” Shah explains.

In addition, the typical approach in batch processing involves analysis of a small discrete sample number, with reference standards bracketing the samples and system suitability tests performed prior to analysis, according to Heise. Rotating through multiple identical analyzers would allow continuous monitoring, but the solution would be costly. Validation of both system suitability tests and the analytical methods for continuous control/monitoring will need additional work to demonstrate that results do not require bracketing with reference standards and that the detection methods do not drift or get poisoned over time when operating for 90+ days,” he says. Failure mode mitigation strategies will also need to be developed, for example with respect to system suitability failure.

In addition, Parsons points out that for in-line and on-line analysis, the inherent requirements of sensitivity and specificity for analysis of process residuals suggests that many of the current and potential online methods would have limitations and may perhaps be able to best provide supportive general information, rather than specific quantitation of residuals. “Offline analysis using traditional validateable techniques might still be required for specific and sensitive quantitation of the process residuals. Nevertheless, if a continuous process can be shown (perhaps by an online monitoring method) to be robust, then reduced sampling of the continuous process for quantitation of residuals may be justifiable,” he notes.

In fact, because continuous processes are expected to operate under steady-state conditions, control through predictive modeling or by exception should be possible, Heise adds. “Multi-variant analysis during development may identify simple in-line monitoring techniques that can control the process to ensure residual impurities do not pass through the whole process. However, the dynamics of the process will be different at the outset until the steady state is reached. A testing strategy defining the frequency of measurement pre- and post-steady state will be required to identify when process equilibrium has been reached and when it begins to fail,” he observes.

“Ideally,” Heise continues, “we don’t really want to be testing anything real-time and on-line. Instead, we want to have confidence that the process will deliver consistently over a defined time scale.”

When moving from batch to continuous processing, similar offline analytical methods may be used. Significant development is needed, however, before on-line/in-line analysis of residual impurities can be widely implemented.

Parsons does note that low-field NMR is a potentially promising technique for on-line analysis of process residuals. “The system is simple to operate, potentially applicable to both batch and continuous processes, and could be performed by stop-flow analysis with direct connection of the flow path to the downstream purification process,” he says. Attractive features of NMR in this regard are its inherently quantitative response factor and its relatively high resolving power, and hence higher specificity than a technique such as UV, while drawbacks include barriers to implementation of a conceptually complex analytical technique and limitations on sensitivity.
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Catalytic Site Analysis and Characterization of a Solvent-Tolerant Aldo-Keto Reductase

WEI JIANG, RUI PEI, WEILIANG WU, PANPAN ZHAO, LIBING TIAN, AND SHU-FENG ZHOU

ABSTRACT
Chiral alcohols are important intermediates of various drugs. Compared with traditional chemical methods, the biocatalytic methods used for the synthesis of chiral alcohols exhibit many advantages, such as mild conditions and high enantioselectivity. Aldo-keto reductases are regarded as promising enzymes that can be potentially applied in the biocatalytic synthesis of chiral alcohols. In this study, a novel aldo-keto reductase, AKR7-2-1, was cloned and purified, and its important conserved sites were analyzed. In addition, this study analyzed the catalytic potential of AKR7-2-1. The optimum reaction conditions were studied; AKR7-2-1 showed excellent thermal stability and pH stability even when the temperature reached 80 °C or pH reached 9.0. Furthermore, AKR7-2-1 has strong enzymatic activity when 11 ketone-containing compounds are used as substrates, indicating the broad substrate spectrum of the enzyme. Most importantly, AKR7-2-1 has superior organic solvent tolerance even in an organic solvent of 30% volume per volume (V/V) or 10 hours in a 10% V/V organic solvent, where 60% enzyme activity was retained. It is worth mentioning that AKR7-2-1 can catalyze the reduction of N,N-dimethyl-3-keto-3-(2-thienyl)-1-propanamine to (S)-N,N-dimethyl-3-hydroxy-3-(2-thienyl)-1-propanamine, an intermediate of the antidepressant drug duloxetine. All this shows that AKR7-2-1 has broad application prospects in the field of biomedicine.

Chiral alcohols are a class of compounds having a hydroxyl group attached to a chiral carbon atom. The compounds are structurally stable and are important intermediates for the synthesis of chiral drugs, perfume flavors, and agricultural chemicals (1, 2). Compared with traditional chemical methods, biochemical synthesis provides a promising strategy for the production of chiral alcohols with the advantages of high efficiency and enantioselectivity and mild reaction conditions. Enzymes and microorganisms are used to catalyze the production of various enantiomerically pure alcohols (3).

The aldo-keto reductase (AKR) is a class of nicotinamide adenine dinucleotide cofactor (NAD(P)H)-dependent enzymes belonging to the oxidoreductase family (4). AKRs are widely found in nature, such as bacteria (5), plants (6), and animal tissues (7). AKRs show great application prospects in the field of biomedicine due to their ability to catalyze the reaction of prochiral ketones to form chiral alcohols. AKR, for example, can be used for the asymmetric reduction of ethyl 4-chloro-3-oxobutanoate (COBE) to ethyl (R)-4-chloro-3-hydroxybutanoate (CHBE), known as an important intermediate in organic synthesis, which can be applied in synthesis of L-carnitine, HMGC0A reductase inhibitor (8, 9). Catalytic reduction of ethyl 2-oxo-4-phenylbutanoate with Bacillus subtilis-derived AKR is an
important way to obtain ethyl (R)-2-hydroxy-4-phenylbutanoate, which can be used as an intermediate for anti-hypertension drugs (10). Only a limited number of AKRs have been obtained and applied to the synthesis of chiral alcohols, however. Five AKRs were cloned for highly stereoselective reduction of bulky ketones by C. Liang et al. through genetic mining from microorganisms such as Candida albicans (CaCR), Saccharomyces cerevisiae (ScCR), Kluyveromyces marxianus (KmCR), and Candida parapsilosis (CPR-C1, CPR-C2) (11). X. Luo et al. cloned an AKR from Kluyveromyces lactis XP1461, which can be used for the synthesis of chiral alcohols (12).

Y.H. Ma et al. cloned a stong heat-resistant AKR from thermophilic bacteria, Tm1743 (13). C. Ning et al. cloned three kinds of AKRs from Lodderomyces elongisporus—LEAKR 48, LEAKR 49, and LEAKR 50—which can be applied to synthesize ethyl 4-chloroacetoacetate (14). In addition, AKR from S. cerevisiae, YOL151W, can be used for asymmetric synthesis of (S)-3-chloro-1-phenyl-1-propanol (15). The new AKR from L. elongisporus, NRRL YB-4239, can be used to synthesize ethyl (R)-4-chloro-3-hydroxybutanoate (16). So far, chiral compounds have been among the top 10 drugs in history, and it is expected that by 2020 chiral compounds will still dominate the best-selling drug list (17). Although the demand for chiral drugs in the medical field is extremely large, there are still few studies on biocatalytic synthesis of chiral alcohols, which cannot meet the demand for chiral drugs in the medical field. Therefore, it is necessary to continue to excavate new types of AKRs to meet the synthetic needs of chiral drugs.

In this study, a novel AKR (AKR7-2-1) was cloned from Bacillus megaterium. The catalytic performance of AKR7-2-1 was subsequently investigated. The three-dimensional structure and important sites of AKR7-2-1 were also analyzed. The extensive substrate profile of AKR7-2-1 and strong organic solvent tolerance indicate the great potentials in the synthesis of high-value chiral alcohols.

MATERIALS AND METHODS

Strains, vectors, chemicals

Escherichia coli (E. coli) DH5α and BL21 (DE3) were cultured in Luria-Bertani (LB) medium. They were used for cloning and heterologous expression. Plasmid pET-28a was used in this study. All enzymes used in this work were from TaKaRa Co., Ltd. (Dalian, China). Plasmid Mini Kit I (200) and Gel Extraction Kit (200) were purchased from OMEGA Co. (United States). Nicotinamide adenine dinucleotide phosphate (NADPH) was purchased from Sigma-Aldrich Co. (Shanghai, China). All other chemicals were purchased from Aladdin (Germany). All chemicals used were chromatographically pure or analytical grades and therefore required no further purification in use.

Amino acid sequence analysis and 3D modeling

The National Center for Biotechnology Information (NCBI) basic local alignment search tool (BLAST) is commonly used in the analysis of bioinformatics (18). The amino acid sequence of AKR7-2-1 was blasted in NCBI, and nine sequences from other sources having similarity to the sequence of AKR7-1 were selected for multiple sequence alignment analysis. The AKR7-2-1 was constructed in a fully automated protein structure homology-modeling server (SWISS-MODEL) commonly used in the construction of three-dimensional models of proteins (19).

Cloning, expression, and purification of AKR7-2-1

The gene of AKR7-2-1 was amplified by bacterial liquid polymerase chain reaction (PCR), and the primer used in this process was synthesized by Bioengineering Biotechnology (Shanghai) Co., Ltd. The DNA fragment of AKR7-2-1 was cloned into the pET28a vector by genetic engineering, and the recombinant vector pET-28a-akr7-2-1 was transformed into E. coli BL21 (DE3) cells for heterologous expression. The AKR7-2-1 was heterologously expressed, and 200 mL of LB medium was added with kanamycin at a concentration of 100 μg/mL. The temperature was set to 37 °C and the cells incubated at 200 rpm. E. coli BL21(DE3) was cultured for three to four hours until the optical density (OD) value reached 0.6–0.8. After that, the temperature was changed to 18 °C, and isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to the culture medium with a final concentration of 0.1 mM. The E. coli BL21(DE3) cells were further cultured for 14 hours. Afterwards, E. coli BL21(DE3) cells were collected by centrifugation and washed three times with phosphate buffered saline (PBS). A sonicator was then used to lyse the cells, and the broken cell debris were centrifuged at 12,000 rpm for 30 minutes. The supernatant was collected and stored at 4 °C for further use. (The previous experimental steps were all performed on ice at all times). AKR7-2-9 was then purified using an AKTA Prime system equipped with a 10-mL Ni-IDA column (GE Healthcare, US). The purification results were subsequently detected using a 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) gel. Finally, the protein concentration of AKR7-2-1 was measured using a protein assay kit (Bradford Protein Assay Kit, Sangon Biotech [Shanghai] Co., Ltd.).

Enzyme activity assays

The total volume of the reaction system was 220 μl, which contained 10 μl of methyl pyruvate, 170 μl of PBS, 10 μl of NADPH, and 30 μl of enzyme AKR 7-2-1. One unit of AKR 3-2-9 was defined as the amount of enzyme that catalyzed the oxidation of 1 μmol NADPH per min. The rates of reduction were assayed at 37 °C by measuring the
change in absorbance of NADPH at 340 nm (ε = 6.22 mM$^{-1}$ cm$^{-1}$). Apparent $k_{cat}$ and Michaelis-Menten constant of AKR7-2-1 (Km) were calculated by using the Lineweaver-Burk double reciprocal plot.

CHARACTERIZATION OF THE AKR 7-2-1 ENZYME

Substrate specificity of AKR7-2-1

The substrate specificity of AKR7-2-1 was investigated under the standard assay conditions by using 11 ketone-containing compounds as substrates. The total volume of the reaction system was 220μl, in which the substrate, PBS buffer, cofactor NADPH, and AKR7-2-1 were set to 10 μL, 170 μL, 10 μL, and 30 μL, respectively. The maximum activity of AKR3-2-9 was defined as 100%. In this experiment, 11 substrates were used: 3-methylcyclohexanone, methyl pyruvate, phenoxyacetone, ethyl levulinate, 2-octanone, acetyl, acetone, 5-methyl-2-hexanone, 4-methyl-2-pentanone, phenylmethylketone, N-Boc-3-piperidone, and N,N-Dimethyl-3-keto-3-(2-thienyl)-1-propanamine (DKTP).

Effect of temperature and pH

Methyl pyruvate was used as a substrate to study the effect of temperature and pH on the reaction. To study the optimum temperature of AKR7-2-1, a gradient was set at a 2°C interval between 30 °C and 50 °C. The reaction system without coenzyme NAPDH was incubated at the corresponding temperature for five minutes. After the completion of the incubation, NAPDH was added to rapidly measure the residual activity of AKR7-2-1 using a microplate reader. To determine the temperature stability of AKR7-2-1, the enzyme was allowed to stand at the corresponding temperature for one hour. After one hour, the residual activity of AKR7-2-1 was determined under standard methods.

To study the optimum pH of AKR7-2-1, CH$_3$COOH-CH$_3$COONa buffer with pH of 4.0–6.0, NaH$_2$PO$_4$-Na$_2$HPO$_4$ buffer with pH of 6.0–8.0, and Tris-HCl buffer with pH of 8.0–9.0 were used. The maximum enzyme activity was defined as 100%. Meanwhile, the pH stability of AKR7-2-1 was investigated by incubating it for 24 hours in the buffer with different pH ranges as described previously. After the incubation was completed, the residual enzyme activity of AKR7-2-1 was determined according to a standard measurement method. The maximum enzyme activity was defined as 100%.

Organic solvent tolerance of the AKR7-2-1 enzyme

Six industrially common organic solvents—including methanol, ethanol, isopropanol, ethyl acetate, acetonitrile, and dimethyl sulfoxide (DMSO)—were selected to study the organic solvent tolerance of AKR7-2-1. The concentration of organic solvents ranged from 10% to 30% V/V. The residual enzyme activity of AKR7-2-1 under different conditions was then determined according to standard methods. In addition, the organic solvent stability was also studied. The above six organic solvents were separately added to the reaction system. The concentration of organic solvent was set at 10% V/V. AKR7-2-1 was placed in the system (without NAPDH) in a 4 °C refrigerator for 10 hours. Every two hours, NAPDH was added to the reaction system and its residual activity was quickly determined by standard methods. The maximum enzyme activity was defined as 100%.

RESULT AND DISCUSSION

Gene cloning and sequencing analysis of AKR3-2-9

In the present study, the cloned DNA fragment of AKR7-2-1 was 963 bp in length. The DNA sequence was translated into an amino acid sequence with a length of 320 bp, which was then subjected to BLAST in NCBI. Nine sequences with different similarities were selected, and multiple sequence alignment
analysis was performed. The result is shown in Figure 1. AKR7-2-1 is 97% similar to aldo/keto reductase from *Bacillus megaterium* (NO.:AUO10603.1), 96% similarity to aldo/keto reductase from *Bacillus aryabhattai* (NO.:WP_088048797.1), 76% similarity to aldo/keto reductase from *Fictibacillus enclensis* (NO.:WP_061968434.1), 73% similarity to aldo/keto reductase from *Ktedonobacterales bacterium Uno11* (NO.:GCE21280.1), 68% similarity to aldo/keto reductase from *Youngiibacter fragilis* (NO.:WP_023384671.1), 64% similarity to aldo/keto reductase from *Sporolactobacillus laevolacticus* (NO.:WP_100488283.1), 58% similarity to aldo/keto reductase from *Paenibacillus cellulosilyticus* (NO.:WP_110042924.1), 57% similarity to aldo/keto reductase from *Paenibacillus lautus* (NO.:WP_096776379.1). In addition, in the N-terminal and middle (blue box, see Figure 1) of the amino acid sequence of AKR7-2-1, a glycine-rich sequence was present, indicating that this motif is highly conserved. The amino acid sequence of AKR7-2-1 also has four highly conserved catalytically active sites located at Asp48, Tyr53, Lys88, and His129 (20). So far, the specific mechanism of action for catalytically active tetrads is not well understood. A limited number of studies have shown that Lys (21) is stable in binding to the coenzyme NAD(P)H by enhancing the stability of the charge interaction and participating in the catalytic reaction; Tyr (22, 23) contains a phenolic hydroxyl group, which binds to the substrate and participates in the catalytic reaction; His (24) is a proton donor in the catalytic process and also relevant to the reduction reaction in combination with the substrate; and Asp (25) plays a vital role in the spatial conformation of proteins.

**Expression and purification of AKR7-2-1**

After induction with 0.1 mM IPTG, the recombinant vector pET-28a-akr7-2-1 was successfully heterologously expressed in *E. coli* BL21 (DE3). The sonicated crude enzyme solution was purified. The SDS–PAGE results can be seen in Figure 2.

**Substrate specificity**

To detect the substrate specificity of AKR7-2-1, 11 substrates with ketone groups were selected in this study, and the results shown in Figure 3 demonstrate the different degrees of catalytic activity on these substrates by AKR7-2-1. Among them, AKR7-2-1 exhibited the highest catalytic activity when methyl pyruvate, which is an important raw material in pharmaceutical production, is used as a substrate (26). When acetylacetone and DKTP were used as substrates, the catalytic activity of AKR7-2-1 is also relatively high. It is worth mentioning that the reduction of DKTP to DHTP is a key step in the synthesis of the antidepressant duloxetine (26, 27). These results indicate that AKR7-2-1 has a good application prospect in the field of biomedicine because of its broad substrate spectrum.

**Effect of temperature and pH**

Temperature and pH are important factors influencing enzyme activity, and their effects on AKR7-2-1 were investigated in this study (see Figure 4). Figure 4A shows that the optimum temperature for AKR7-2-1 is 37 °C, at which point enzyme activity was the highest. Enzyme activity was shown to increase at 30 °C and then decrease as the temperature approached 50 °C. Further, enzyme activity slowly decreased as the temperature exceeded 37 °C, but still retained 70% or more relative activity even when it reached 50 °C. Previous studies by other researchers had reported an optimum temperature of 30 °C for an aldo-keto reductase cloned from *Kluyveromyces lactis* XP1461 with enzyme activity decreasing sharply when the tempera-

![Figure 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis of expression products. Lane M = marker; Lane 1 = not induced with isopropyl β-D-1-thiogalactopyranoside (IPTG); Lane 2 = crude enzyme solution after IPTG induction; Lane 3 = purified AKR3-2-9.](image-url)
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The reductase in previous studies kept less than 20% activity when the temperature was set to 50 °C (12). Compared with the aldo-keto reductase in previous studies, AKR7-2-1 was well resistant to high temperature. The thermal stability of AKR7-2-1 was investigated by incubating the enzyme at a temperature range of 30 °C to 80 °C (Figure 4B). It was shown that relative enzyme activity decreased with the incremental increase in temperature; however, more than 70% activity remained when the temperature reached 80 °C, showing superior thermal stability. In comparison, an aldo-keto reductase cloned from Lodderomyces elongisporus NRRL YB-4239 by Q. Wang et al. completely lost activity when incubated for 30 minutes at 45 °C (28).

As can be seen in Figure 4C, the optimum pH of AKR7-2-1 is 6.0. When the pH is lower than 6.0, the enzyme activity of AKR7-2-1 is drastically decreased, with activity falling to 20% when pH is reduced to 5.0. Interestingly, as the pH dropped from 5.0 to 4.0, it decelerated the reduction of enzyme activity. On the other hand, the activity of AKR7-2-1 decreased only mildly when the pH was above 6.0, maintaining 60% activity even when the pH reached 9.0. In addition, there is an interesting phenomenon that even under the same pH conditions, the enzyme activity of AKR7-2-1 in the phosphate buffer was higher than in the acetate buffer or the Tris-HCl buffer. This is similar to the results studied by Y. Wang et al. (29). AKR7-2-1 exhibited the best stability in a buffer with pH of 6.0, as shown in Figure 4D. Its activity decreased to less than 20% when incubated in buffer at a pH below 5.0 for 24 hours. The relative enzyme activity also decreased at a pH ranging from 6.0 to 9.0, although it still retained 40% activity when the pH reached 9.0, which indicates an ability to resist extreme pH. In a similar study by Q. Wang et al. (28), the enzyme retained less than 30% of activity even when it...
was only incubated in a buffer of pH 8.0 for 60 minutes.

**Kinetic analysis**

In this study, the kinetic analysis of purified AKR7-2-1 was also performed. The results of various kinetic parameters of AKR7-2-1 show that $V_{\text{max}}$, $K_m$, $K_{\text{cat}}$, and $K_{\text{cat}}/K_m$ of the enzyme was 110.0 uM/min, 4.380 uM, 57.29 min$^{-1}$, and 13.04 min$^{-1}$uM$^{-1}$, respectively. These kinetic parameter data indicate the strong binding ability to the substrate (methyl pyruvate). In addition, the $K_{\text{cat}}/K_m$ of AKR7-2-1 is 13.04 min$^{-1}$uM$^{-1}$ (218 s$^{-1}$mM$^{-1}$), demonstrating an extremely high catalytic efficiency compared to other reported $K_{\text{cat}}/K_m$ of AKRs. For example, the $K_{\text{cat}}/K_m$ of kmAKR-W297 is 60.97 s$^{-1}$mM$^{-1}$ (29), Tm1743 is 54.6 s$^{-1}$mM$^{-1}$ (13), and CgKR1-F92L is 33.2 s$^{-1}$mM$^{-1}$ (30).

**Detection of AKR3-2-9-tolerant organic solvent properties**

Enzyme catalytic systems containing organic solvents have many advantages in industrial production. Many natural enzymes, however, are poorly resistant to organic solvents (31). As shown in **Figure 5A**, methanol, ethanol, isopropanol, ethyl acetate, acetonitrile, and DMSO were used to study the organic solvent tolerance of AKR7-2-1, which was able to survive to 10% V/V of organic solvents with more than 80% residual activity. When the concentration of the organic solvent in the enzymatic reaction increased from 10% V/V to 30% V/V, the enzyme activity of AKR7-2-1 began to gradually decrease but still retained more than 60% activity.

Furthermore, the organic solvent stability of AKR7-2-1 was also studied by incubating the enzyme in methanol, ethanol, isopropanol, ethyl acetate, acetonitrile, or DMSO with the concentration of 10% V/V for 10 hours, and the enzyme activity of AKR7-2 was detected every two hours. As shown by **Figure 5B**, AKR7-2-1 retained more than 50% enzyme activity in DMSO after 10 hours of incubation, while it retained approximately 40% activity in methanol, ethanol, and acetonitrile under the same reaction conditions. An aldo-keto reductase cloned by X. Luo et al. from Kluyveromyces lactis XP1461 was only able to tolerate up to 5% V/V of organic solvents such as isopropanol and ethyl acetate (12). In addition, an aldo-keto reductase (Tm1743) cloned from Thermotoga maritima has been reported to withstand up to 10% V/V of organic solvent (13). Compared with the aldo-keto reductase reported, AKR7-2 showed superior organic tolerance.

**Molecular modeling**

A three-dimensional model of AKR7-2-1 that was constructed using SWISS-MODEL, AKR7-2-1, and the Crystal structure of the *E. coli* Tas protein (SMTL ID: 1lqa.1) were shown to be structurally similar (Figure 6A). This protein is an NADP(H)-dependent aldo-keto reductase (32). The amino acid sequence of AKR7-2-1 is compared with the amino acid sequence of 1lqa.1 as shown in Figure 6B. The homology modeling results of AKR7-2-1 illustrated that it may have a binding site for NADPH coenzyme with a high conservative. As shown in Figure 6B, the amino acid positions marked with white letters on a red background are possible binding sites for NADPH and AKR7-2-1.
CONCLUSION

In this study, a novel aldo-keto reductase AKR7-2-1 with conservative site was excavated. The broad substrate spectrum showed that the enzyme was especially able to catalyze DKTP to DHTP, an important precursor of the antidepressant drug duloxetine. In addition, the enzyme was shown to have superior thermal stability, pH stability, and excellent tolerance to a wide range of organic solvents. These characteristics make AKR7-2-1 a promising enzyme to be applied in the medicine field.

ACKNOWLEDGMENTS

The work was supported by the Natural Science Foundation of China (No. 21808073), the high-level personnel activation fee of Huaqiao University (No. 600005-Z17Y0072), and Quanzhou City Science & Technology Program of China (No. 2018C008).

AUTHOR CONTRIBUTIONS

Rui Pei did experiments, Weiliang Wu, PanPan Zhao, and Libing Tian provided help for the experiment. Pei and Wei Jiang wrote and modified the article, and Jiang and Shu-Feng Zhou designed and supervised the work.

CONFLICT OF INTEREST

The authors state that they have no competing interests.

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Removing Gaps in Data Integrity

FDA guidance is expected to improve industry practices, but work is also needed to bridge disparate industry and software engineering standards.

AGNES SHANLEY

Over the past few years, after some notable failures at a number of companies (1), global regulators have been paying much closer attention to how pharmaceutical manufacturers safeguard the integrity of their data, to prevent accidental manipulation as well as fraud. Data integrity is the foundation of the current good manufacturing practices (cGMPs). According to FDA, between January 2015 and May 15, 2016, 21 out of the 28 warning letters issued to pharmaceutical manufacturers centered around problems with data integrity (2).

As Orlando López, Kansas-based senior site automation specialist for a Big Pharma company, has noted (3), connections between different data points (i.e., initial data capture, metadata, and records) cannot be weakened or broken because they provide the only objective evidence that operations meet regulatory requirements and are being managed responsibly. These intact connections are also needed for process validation and continuous process verification.

FDA’s 2016 draft guidance clarified a number of best practices, such as the need for paper and electronic recordkeeping to use the same basic practices, and for an administrative role for managing data, separate from functions that are generating and recording that data. FDA also introduced the ALCOA acronym to emphasize the fact that data must be “attributable, legible, contemporaneous, original (or a true copy), and accurate.”

In December 2018, FDA updated this guidance (4). “The new guidance serves to clarify data integrity requirements that have consistently challenged organizations. It firms up expectations around data/metadata and the data lifecycle within a risk-based structure of systems and design controls,” says Kir Henrici, CEO of the Henrici Group, a consultant for the Parenteral Drug Association (PDA), which published best practices on laboratory data integrity in 2018 and plans to publish manufacturing data integrity guidance by the end of 2019.

FDA has emphasized the need for careful risk management, the importance of audit trails and access controls, and the need to save all records that could be relevant to cGMP compliance. Its latest data integrity guidance also

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underscores the importance of senior management support and involvement in data integrity efforts. “Leadership and culture are key, and, if underdeveloped, are the starting points for compliance risks and gaps,” notes Henrici.

Training and ongoing communication with senior management are crucial to establishing and maintaining this support, says López. Expressing potential losses or liabilities in dollars and cents terms can be especially effective, he says, noting a 2017 hacking incident at Merck & Co., which cost $105 million to fix (5) and might have been prevented if investment had been made in data security. He also recommends keeping senior management aware of regulatory citations in a way that allows them to connect data issues to lost batches, product recalls, incorrect labels, or formulation changes. “Senior managers need to hear about money. It gets their attention immediately,” he says.

RISK-BASED APPROACH

It is also important to take a systematic, risk-based approach to data integrity programs, says Henrici. Some organizations may roll out data integrity programs in fragments, or as a reaction to a specific event, but then allow efforts to trail off, she says. “Pharmaceutical companies need to structure a systematic, risk-based approach that integrates the quality management system (QMS), which assures the efficacy and sustainability of the entire data integrity initiative.”

Process mapping is especially important in ensuring that the right documentation is easily accessible to staff, but also to regulators. “Companies should track their ‘quality decisions’ to identify where non-cGMP records (e.g., emails) will be required to demonstrate cGMP compliance,” says Henrici.

“You need to understand each specific process. Then, if you do a good risk assessment, to record which systems and which data you will focus on, you will be in a much better position to demonstrate full cGMP controls to the authorities,” says López. “If you have done both the risk assessment and the process mapping correctly, there should be no doubt that you are implementing controls to the correct records,” he says. Decisionmaking processes must be documented, says Henrici—not only reported data but any data that support quality decisions—and they need to be justified, retrievable, and reviewable, she says. Maintaining a clear audit trail and using the best approaches to computer system validation will be crucial and will require new training and staffing practices, says Henrici. “Next-generation quality assurance will consist of multiple disciplines or working teams to keep pace with data integrity requirements in the era of smart manufacturing, big data management, and artificial intelligence,” she says.

GETTING TO A DEEPER LEVEL

In a sense, however, data integrity is only the first step in a continuum. “Pharmaceutical industry guidelines often refer to data reliability, which is a step beyond data integrity,” says López. “But, ultimately, the goal for our industry is data quality, which is a step beyond reliability. We cannot reach that point with the guidelines that we have now,” he says. “And cGMP requirements alone will not suffice.” (6) Another challenge is the fact that existing pharmaceutical industry data guidelines don’t go deep enough, to the engineers and IT professionals who are implementing the systems, says López. “ALCOA may give them the ‘why,’ but it doesn’t give them the ‘how,’” he says.

Annex 11, first published in 1986, addresses data integrity in a more explicit way than the US 21 Code of Federal Regulations (CFR) Part 11, he notes. López recalls one Big Pharma company that correlated Annex 11 with Part 11 computer system requirements, which made things easier for engineers and IT professionals implementing new platforms.

ALCOA addresses the ‘why’ but not the ‘how’ of data integrity, leaving questions for IT professionals and engineers implementing new platforms.

Currently, there are a number of different data integrity guidelines. In pharma, these include regional ones as well as those developed by professional societies such as PDA and the European Compliance Academy (ECA). In addition, the software industry has codes of its own, as does the manufacturing industry in general. The focus of each of the relevant pharma guidelines is different, López says, and the definition of data integrity in software engineering standards (i.e., as mainly about data security and not changing the records), differs somewhat from its definition in the pharmaceutical industry. The result can be confusing to IT professionals, at least initially, and take time to sort out.

“I would love to see integration between pharma standards and the software engineering standards, because currently the industry is not speaking the same language as software development academics. For example, for the pharma industry, validation is a process associated with the system lifecycle (SLC), which continues from the start of a project until decommissioning. For software development, validation is just the testing phase of the SLC. So when you hire a
new graduate, he or she won’t understand the difference,” says López.

Without understanding the pharma validation concept and the relationship between software engineering and software quality, people in pharma’s IT and engineering departments will have different understandings and the resulting programs will be incomplete, he says. López recalls seeing such a situation when asked to consult for one mid-sized pharmaceutical company several years ago. Data security had been made part of the system’s user requirements, but, as one looked at the different processes throughout the product lifecycle, there was one point where the computer system was missing provisions for security, so that anyone could make changes to the data. “There was testing, but no transparency, and in the design document, there had been no steps taken to ensure security throughout the lifecycle,” he says.

Common standards and definitions will be the key to moving the industry beyond data integrity to data quality, López says, explaining that the origin of the International Society for Pharmaceutical Engineers’ (ISPE’s) GAMP 5 guidance (7) is the International Standards Organization’s (ISO’s) 9000-3, which is also the foundation of the pharmaceutical industry’s guidelines. “Why are we speaking different languages?” he asks. “We need to synchronize all the different standards (e.g., ISO, ISPE, The Institute of Electrical and Electronics Engineers [IEEE]), and pharma’s so that we are speaking one common language and so that we will understand each other,” says López. Without that common understanding, gaps will persist, some worse than others, he says.

The pharmaceutical industry’s approach to data integrity and quality will need to evolve as the industry moves to a Pharma 4.0 model and adopts data analytics and artificial intelligence. At INTERPHEX 2019 in April, Henrici spoke of the need for a “Data Integrity 4.0” strategy, a framework for matching IT and data integrity rules. She also noted that FDA approved 12 artificial intelligence algorithms in 2018.

If the industry is to reap the benefits of using algorithms and artificial intelligence, she said, companies will need to create multifunctional data governance teams to bring different perspectives to this effort, and facilitate communication between industry and regulators, and data will need to be safe, secure, and relevant (8).

Common standards and definitions will be the key to moving the industry beyond data integrity to data quality.

In the end, the required knowledge is already there for implementing new IT approaches, says López, who developed an analytics and business intelligence platform at a Big Pharma company several years ago, before the term “Big Data” had even become a buzz phrase. He advises breaking the problem down to its simplest level: the data wave and input/output (I/O). “With a Big Data project, instead of one, you might have 20 data waves and I/Os. The pharmaceutical industry has been working on all the technologies that industries are exploring (e.g., wireless, industrial internet of things, and Big Data). In the end, each type involves software and hardware, input and output, and there are certain tools best suited for each situation,” he says. “On the fundamental level, we need to understand the relationship between the system, process mapping, data wave sets, and intended use, and simply apply what we have learned during the past 30 years to the new technologies,” adds López (9).

The industry has come a long way in improving data integrity since the Barr decision in 2005 (1), a landmark case that resulted in mandates for recordkeeping and the investigation of out-of-specification conditions. New guidance and better integration of existing best practices—not only those designed for pharma’s end users, but for implementation specialists in IT and engineering—promised to push pharmaceutical manufacturers beyond data integrity in the future.

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W ith viruses playing a greater role in everything from vaccine development to gene therapy, ensuring their rapid, accurate, and biologically relevant enumeration isn’t just an analytical “plus”; it’s the first step in the development of biological tools that support human health.

The catch? Traditional virus-quantification methods haven’t always cleared the high bar that contemporary needs set. That began to change a few years back when a startup in Boulder, CO, designed a new platform for rapidly enumerating total virus particles using reagent technologies that are both biologically relevant and biologically specific.

Seeing the potential in this emerging technology—called the Virus Counter platform—Sartorius Stedim Biotech acquired it in 2016 and made it even better, engineering it into a robust commercial platform. We sat down with Antje Schickert, product manager for virus analytics at Sartorius Stedim Biotech, to learn how the Virus Counter makes even the trickiest virus enumerations…well, count.

BioPharm International: Which industries have a need for virus quantification?
Schickert: Virus enumeration plays a critical role in vaccine development and production, viral vector manufacturing, and many other processes where the viruses are often the final product. The increasing demand for viral vectors, requires more efficient manufacturing processes to keep pace with the needed quantities of viruses.

Optimization of those manufacturing processes commands a very detailed understanding of how the concentration, and also the quality, of the product—which, in this case, is a virus—is influenced by different parameters such as different growth conditions or purification steps.

In applications such as gene therapy, viruses are often directly administered to patients as a therapeutic, and in this case, it’s really vital to characterize these viral therapies in detail to ensure the safety of the patient. Enumeration and a very good understanding of the composition of these products are part of that in-depth characterization.

In the past, the availability of quick and reliable quantification methods often failed to effectively provide these important insights.

BioPharm International: How did these industries traditionally enumerate viruses?
Schickert: Traditional methods of virus enumeration include plaque titer assays or TCID_{50}. These are often referred to as functional assays, but they present a drawback in the delay they impose because of the labor-intensive nature of the techniques. Results often aren’t available for days or weeks because we have to wait until the viruses replicate in the cell to actually quantify them. That can slow or pause manufacturing processes, or provide results that might not be as relevant as when the sample was taken.
Many of these traditional methods are also inherently variable, and that can decrease confidence in the results that they deliver. So, these methods really cannot keep pace with the modern requirements of cell and gene therapies.

**BioPharm International:** What are some of the more modern approaches that are used to prevent the delays caused by traditional assays?

**Schickert:** To address the delays that functional assays cause, methods such as PCR and ELISA were more recently introduced into the virus quantification field. These methods are more rapid than more traditional functional assays, but they often rely upon measurements of viral components only such as viral proteins or nucleic acids; then, they calculate the titer from these measurements. That means that results can potentially be biased, as they’re derived values rather than values based upon direct measurements of intact viruses.

**BioPharm International:** How does the Virus Counter address these gaps in the field of virus quantification?

**Schickert:** The Virus Counter platform was designed to measure virus titers directly and with high speed and precision. The readout is biologically relevant because we look at total viral particles that are directly measured using specific binding reagents. That can have a significant impact on, for example, patient safety.

Sartorius offers different reagents to address diverse customer needs. We have a more universal quantification reagent that has very broad utility and can be used with a large variety of different viruses, but we also offer an antibody-based reagent line that allows virus detection with high affinity and specificity.

We also emphasize the ease of use of our instrument. From system operation to data analysis, everything is software assisted, and that means we limit the risk of any kind of user error or variability.

**BioPharm International:** Can you describe how the Virus Counter platform works?

**Schickert:** The Virus Counter platform was purpose-built to enumerate viruses. As mentioned, the platform includes an instrument, software, and the reagents we’re using to detect viruses. Viruses are directly labeled with either fluorescent dyes or antibody-based reagents. Inside the instrument, the virus sample is guided in a fluidic stream and focused to a small core of moving fluid. A laser is then used to excite the fluorophores that are directly associated with the viruses as they pass the laser. The Virus Counter instrument then detects the emitted light and uses it, together with the sample flow rate, to calculate total particle concentration in the virus sample.

**BioPharm International:** What is the significance of counting total virus particles in a direct manner?

**Schickert:** Virus samples can be incredibly heterogeneous mixes of infectious viruses, nonfunctional particles, and unassociated nucleic acids and proteins that weren’t assembled into virus particles.

Diverse virus quantification methods actually quantify different sub-fractions of the virus sample. For example, plaque assays only enumerate functional particles. Depending on the virus type and the manufacturing process, however, functional particles can actually be a very small fraction of total particles within the sample. ELISA assays usually quantify viral proteins in the sample, and then use that data to calculate a virus titer. PCR quantifies virus-specific nucleic acid sequences in the sample and derives a titer value from that measurement.

So, the heterogeneity of the sample and the nature of these quantification assays are responsible for the diverse results that we obtain with different quantification methods when we measure the same virus sample.

The Virus Counter directly quantifies all intact virus particles by measuring a fluorescent signal—so the readout here is total particles, and it’s really critical for the in-depth understanding of the virus sample composition and for the safety of the patient.

**BioPharm International:** Where do you see the advantages of the Virus Counter technology over existing enumeration assays?

**Schickert:** The Virus Counter platform enables users to measure virus samples in near real time and with very high precision, and that makes Virus Counter results actionable. The technology empowers our users to increase viral vector yields by comparing, for example, different growth conditions and recovery during process development. Users can also track virus titers throughout their process and determine ideal conditions and even detect possible challenges early on in their process. Additionally, the Virus Counter platform increases safety and efficiency by facilitating the in-depth characterization of virus samples and increases the understanding of the total particle concentration in the final product.
New and updated technologies have been released over the past few months for a range of bioprocessing tasks to spark innovation and support efficiency. The following are a sampling of such products.

**CELL CULTURE MICROBIOREACTOR SYSTEM**
A new generation of the ambr 15 cell culture automated microbioreactor system from Sartorius Stedim Biotech (SSB) offers increased flexibility and expanded capability for clone selection, media and feed optimization, and early process development work (1).

The Generation 2 system replicates laboratory-scale bioreactor performance at the 10–15 mL microscale and controls up to 48 single-use bioreactor cultures in parallel. The updated design incorporates new features to improve process flexibility, expand the system’s functionality, and allow more applications to be investigated.

New features include a one-year license of SSB’s clone selection software, which provides simplified, streamlined multivariate data analysis for faster, more consistent cell-line screening and ranking, according to the company. Using the system’s new functionality, election of clones, media, and feeds can be performed under perfusion mimic conditions to bleed large volumes of culture and quickly remove spent media from the microbioreactors. The company states that a new flexible workstation layout and an expanded tip bin capacity provide greater operator walk-away time.

New culture passage steps and rapid vessel drain functionality allow for the adaptation of cell lines to different media for media screening studies to be performed in the microbioreactors. New media mixing steps automate the creation of media blends, eliminating the need to pre-mix. Clone stability studies can also be set up with serial passages performed and fully automated in the microbioreactors. Rapid vessel drain functionality for automated cell passaging and media exchanges in the microbioreactors supports cell and gene therapy applications. New culture station design provides lower stirrer speed control suitable for more sensitive cell lines.

The system also provides a robust screening platform for development of cell and gene therapy processes, including HEK293 for viral vector production, T-cells, induced pluripotent stem cells, and other immune-derived cell lines.
AUTOMATION SOFTWARE FOR CELL THERAPY

The Chronicle web application, the next generation of GE Healthcare’s myCryochain software, now supports the complete cell therapy workflow (2). Chronicle automation software is a GMP-compliant, fit-for-purpose digital solution for the optimization of complex cell therapy process development and manufacturing.

Chronicle automation is suited to increase efficiency while meeting regulatory compliance using real-time supply chain tracking, hardware performance monitoring, SMS or email alarms, and comprehensive electronic batch records. Software capabilities include a unified digital space that monitors all facility manufacturing operations and supply chain logistics with real-time data acquisition and notifications. According to the company, electronic batch records increase productivity, reinforce GMP compliance, and improve the security of patients’ samples through increased traceability.

Built by cell therapy platform solution engineers, the software supports electronic standard operating procedures, which are suitable for specific processes to manage deviations, promote adherence to protocol, and provide guidance to ensure sensitive patient cells are handled appropriately. Additionally, the software integrates with the full range of GE instruments as well as many third-party instruments and has been independently audited against GAMP5, 21 Code of Federal Regulations Part 11, and EU Annex 11.

EXPANDED GASKET RANGE

Watson-Marlow Fluid Technology Group (WMFTG) expanded its BioPure gasket range for high purity fluid management (3). The high-purity gaskets cover a range of applications and support leak resistant connectivity within pharmaceutical and biotechnology production processes, reducing validation risks in contamination-free applications.

According to WMFTG, the gaskets use new materials—polytetrafluoroethylene and a synthetic rubber and fluoropolymer elastomer (Viton, Chemours)—to provide a high level of chemical and steam resistance. Each of the high-purity gaskets have been engineered to deliver improved sealing performances under clamping compression. The BioPure range offers lot traceability on every component, silicone products manufactured and packed in an ISO 14644-1 Class 7 cleanroom, compliance with FDA regulations CFR 21 177.2600, dedicated validation and qualification data to help achieve cGMP requirements, and USP Class VI compliance and animal-derived component free.

IIOT-ENABLED FLOW SENSOR

The Aventics AF2 flow sensor from Emerson continuously monitors air consumption, which allows for rapid leakage intervention in applications such as packaging (4). The sensor continuously monitors air consumption in pneumatic systems, enabling compliance with the energy management standard DIN ISO 50001. The device is one of various Industrial Internet of Things (IIoT) components offered by the company for networked plants in factory automation.

“The Aventics AF2 series flow sensor is ideally suited to those companies aiming to control and optimize their energy usage,” said Andreas Kliewe, expert for energy management with Emerson’s machine automation business, in an April 2, 2019 press release. “The device records the flow rate and compressed air consumption in a system, sending a signal to the controller when pre-set levels are exceeded. This helps avoid excessive energy loss and enables rapid intervention in the event of an issue.”

The company states that the device’s modular construction makes it suitable for installation where space is limited. The sensor is delivered precalibrated with its filter, which can be configured in any maintenance unit. An organic light-emitting diode display on the sensor provides local indication of all relevant operating and diagnostic data. The sensor is available as a normally open and normally closed version. Analog outputs can be switched with a signal from 4 to 20 mA. According to the company, these signals can be interpreted directly by many controllers.

The sensor’s IO-Link capability and the Ethernet interface enable users to communicate with existing controllers. Communications protocols integrated into the device include Open Platform Communications Unified Architecture and Message Queuing Telemetry Transport. A web-based dashboard shows real-time data for the users.

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Cultural and language discrepancies during an audit can be resolved using what many call a “playbook,” says Siegfried Schmitt, PhD, vice-president, technical, Parexel Consulting.

Q: We are preparing for our first inspection by an overseas regulatory agency. This will be a challenge from several perspectives: language, culture, and presentation style. We believe we have covered the first two issues through highly experienced translators and cultural training sessions with coaches. Our concern is about how to present and respond in an appropriate manner. Can you give any advice?

A: Hosting a foreign agency for the first time is always challenging, but as with all inspections, nothing beats preparation, practice, practice, and more practice. Even seasoned personnel can become flustered, nervous, or even unsure how to respond to unfamiliar questions or requests, especially when asked in a foreign tongue. One tool proven to be particularly useful in such situations is a playbook. Playbooks may be known by other terms, but essentially these are aide-memoires that function as references for company representatives who need to present or answer questions from inspectors or auditors.

The playbook may include prepared answers to anticipated questions the auditor may ask and/or detailed information about process steps.

How detailed should these playbooks be and in what format? Here is what has stood the test of time:

- Always start with a succinct, logical, and convincing few lines of text and/or graphics.
- If necessary, have a second playbook with more detailed, supporting information.
- Have a third party (i.e., someone unfamiliar) review your playbook and/or provide feedback.

The first point is the most important. As we all are experts in what we do, we can talk in great detail about it. But inspectors do not have unlimited time. They want to receive answers that provide them with clear and easy-to-comprehend information. This is best illustrated with an example. The inspector may ask: ‘What is your bioburden control strategy for this product?’

Without a playbook, the answer may go like this: ‘In step 5 after vessel 234 on the second floor, remember you saw this reactor on the tour of the facility. We have installed a 0.2-micron filter ...’

With a playbook, the answer would be more similar to this: ‘We have a bioburden control strategy that takes into account environment, process equipment, material handling, product properties, and regulatory expectations. For the five steps produced in our facility, we have prepared a strategy paper, which has subsequently been verified during the validation studies and ongoing monitoring.’

Of course, should the inspector then want to know more details, this is when all the evidence can be presented. Again, it may be useful to have a playbook ready for this, especially if many activities should be organized in a logical sequence and timeline.

Playbooks have several benefits, as they help those responding in an inspection to give clear answers. They also help prepare the site personnel to find answers to difficult questions (e.g., why certain deviation investigations are overdue, or why a particular change hasn’t been documented as per requirements). They also help summarize convoluted event histories or complex situations. Often, one has to overcome obstacles to reach a successful outcome, and playbooks help to focus on the positives, rather than to overemphasize the difficulties experienced.

Then why should one have these playbooks reviewed by someone from the outside? Well, the inspector will be exactly such a person (i.e., an outsider who isn’t necessarily familiar with the terminology, the facility, or the processes used at the site). Therefore, by having an outside third party review the playbook, you essentially perform a dry run of the inspection.

In summary, these playbooks are a useful tool in any inspection or audit, whether foreign or local, but writing these in a clear and concise manner is anything but child’s play.
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