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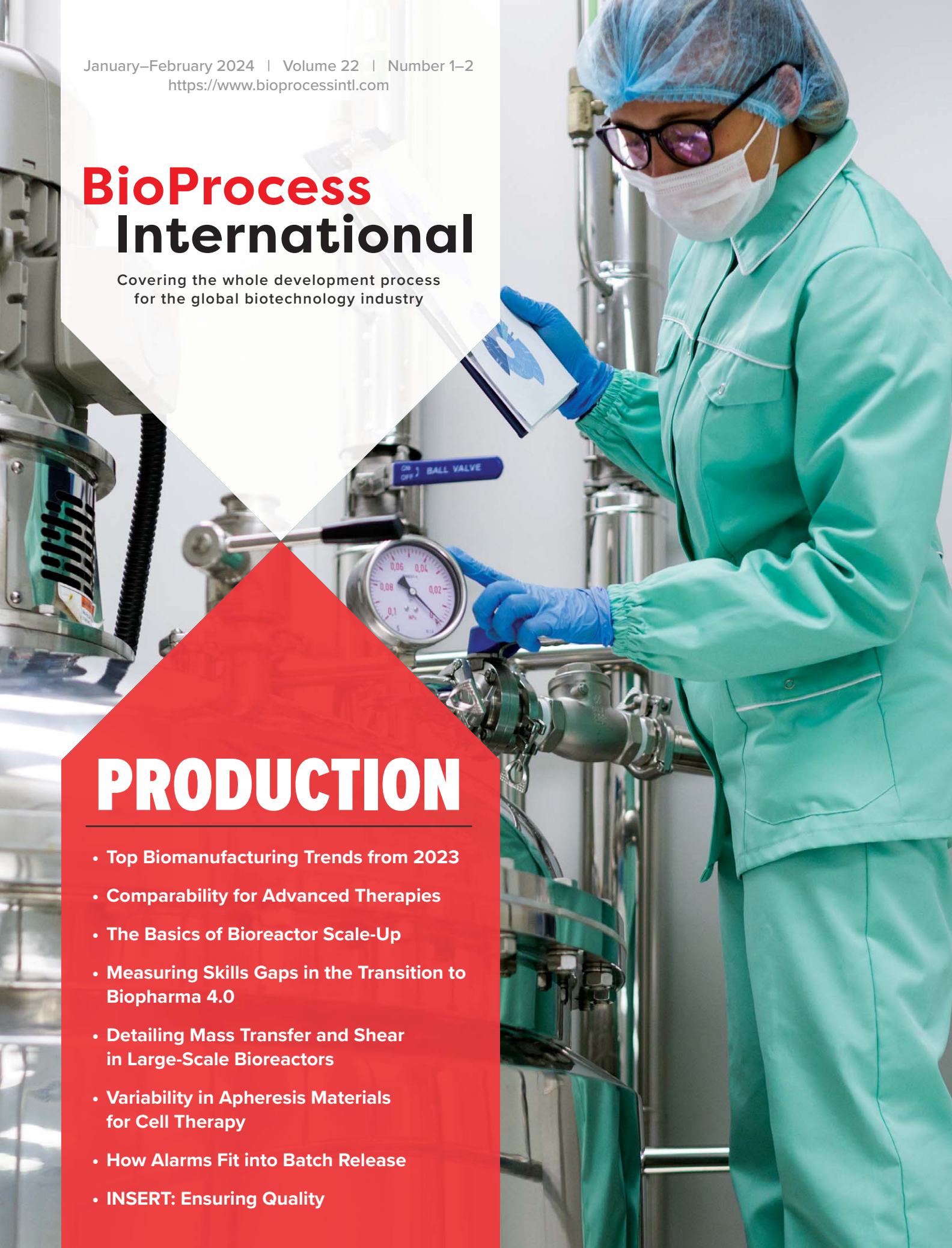
BioProcess International

Covering the whole development process for the global biotechnology industry



PRODUCTION

- Top Biomanufacturing Trends from 2023
- Comparability for Advanced Therapies
- The Basics of Bioreactor Scale-Up
- Measuring Skills Gaps in the Transition to Biopharma 4.0
- Detailing Mass Transfer and Shear in Large-Scale Bioreactors
- Variability in Apheresis Materials for Cell Therapy
- How Alarms Fit into Batch Release
- INSERT: Ensuring Quality

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VOLUME 22 NUMBER 1–2

ON THE COVER
Once a veritable "black box," bioreactor operations increasingly are both knowable and controllable, as illustrated in this image from Adobe Stock (HTTPS://STOCK.ADOBE.COM). *Learn more about this month's BPI contents — both in print and online — on page 2. And find us online at https://www.bioprocessintl.com.*

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https://www.bioprocessintl.com/eBooks

BPI's 2024 eBooks address many topics. In January and February, look for our features on conjugate products and bioprocessing 4.0.

Featured Report: Ensuring Quality

Essential to product safety and efficacy, quality control (QC) lies at the heart of regulatory compliance, process optimization, and drug-product consistency. As this month's **insert** shows, QC depends heavily on analytical technologies and environmental monitoring.

Focus on Advanced Therapies

Every cell/gene therapy (CGT) starts with human cells, the inherent variability of which complicates CGT manufacturing. For consistency in drug safety and efficacy, it is crucial to address such variability and optimize processes at every step – starting even before patient or donor cell collection. As Becky Cap explains on **page 10,** automated apheresis enables efficient isolation of blood-derived starting material for advanced therapies. But it is a time-consuming process with many variables to consider.

Focus on Business

The biopharmaceutical industry has shown great resilience in adapting to economic swings and supply-chain challenges. Technological advances help to improve production efficiency and enable cost-effective bioprocessing around the world. As Erica Friedman shows on **page 12,** significant expansions in some bioprocessing activities during the pandemic created supply-chain pressures, availability challenges, and delivery delays. Those are some of several trends elucidated in the latest BioPlan Associates report.

Focus on Compliance

Comparability studies are needed to support manufacturing process changes through the life cycle of all biologics. Such changes can affect equipment, raw and critical starting materials, manufacturing process scale, and product stability. Especially for cell/gene therapies, the criticality of changes and estimations of how they affect product characteristics determine the amount of comparability data needed. On **page 16,** a report from the 2023 CASSS Cell and Gene Therapy Products Summit describes best practices and regulatory expectations for evaluating comparability of advanced-therapy quality, safety, and efficacy during and after process changes.

An Introduction to Bioreactor Scale-Up

Biomanufacturing processes typically involve scaling up production from miniaturized, high-throughput bioreactors to bench-scale single-use/glass units and then larger pilot- and production-scale tanks. Scaling is an involved task requiring a delicate balance between equipment design and operational capabilities to provide consistent hydrodynamic and mass-transport conditions for cell growth and production. As Muhammad Chaudhry explains on **page 22,** factors contributing to the difficulty of scaling include biological complexities and heterogeneous environments (especially in large-scale reactors) that create substrate and pH gradients and process variability. In this first installment of a series, the author highlights key challenges encountered in production process scale-up.

Oxygen Mass Transfer (OTR) and Shear in Large, Stirred-Tank Bioreactors

Dissolved oxygen (DO) is a key rate-limiting substrate in cell-culture operations. Through sparging and impeller agitation, bioreactors maintain DO concentrations that support cell respiration. Impeller agitation promotes mass transfer of sparged gas bubbles through culture media, and the rate of OTR across the gas–liquid interface is a function of its intrinsic properties and the design and operating parameters of the bioreactor. On **page 38,** authors from AGC Biologics present a study on OTR in a 20,000-L stirred-tank bioreactor. Results from such studies can be used to assess the maximum cell density that a bioreactor can support. The authors also describe a shear-proof design space for operating conditions at that scale.

Tracking Current and Future Skills Gaps for Bioprocess 4.0 Staffing

Digitalization promises increased efficiency, productivity, and quality in bioprocessing. On **page 28,** Beckwith et al. discuss emerging skills required for the "biopharma-4.0" era, highlighting gaps and their causes as well as potential strategies to future-proof the bioprocessing workforce. The authors provide a general analysis of emerging and impending trends in the biomanufacturing sector, highlighting gaps associated with automation, data science, sensor technology, and cyber clusters.

Alarms and Batch Release

As an integral part of biomanufacturing operations, alarm systems could aid in batch release. On **page 56,** authors from PQE Group describe a process flow for including alarm review as part of an overall quality management system.

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FROM THE EDITOR

With Anne Montgomery's retirement this past November, I am now the last founder standing on the BPI staff. It began in 2002 as an idea from Brian Caine and Stephanie Schaffer on the business side with Anne and me on the editorial side, and we put out our first issue in January 2003. Now the brand includes conferences in Boston, San Diego, Europe, and Asia; a training academy; an online news outlet; webinars and custom publishing; and of course the publication that you hold in your hands. And I lost track of the number of people who were closely involved when it went past a couple dozen.

For me, taking over as editor in chief after all that time is absolutely the end of an era and beginning of a new one. For the other editors, it is sure to be a period of adjustment. But for the publication and its overall brand, this is merely the next step in a continual evolution. I'm sure that readers and conference goers will barely notice. Authors and editorial advisors are in for a few (hopefully good) surprises. And some operations will change behind the scenes.

For over 20 years, I've been a process- and product-oriented workhorse — often joking that it was time to put on my blinders, duck down, and plow ahead when things needed to get done. Editor-in-chiefdom forces me to take off those blinders and lift my gaze to see the big picture every day, though I'm doing my best to be "chiefly" in the mornings and remain an editor in the afternoons. That seems to work just fine so far: I'm in the US Pacific time zone, and much of our business seems to happen in the first part of my day anyway. But it does mean that you can expect a more immediate response from emails earlier in the day.

There I go again, getting into the weeds of things. It's where I live when I'm not gardening, doing barn chores, riding my horse, shopping farmstands, or hiking the Oregon countryside with my Labrador. I've learned everything I know about the biopharmaceutical industry — and about business press publishing — on the job and in the thick of things, and thanks to Anne's mentorship along with the invaluable advice from our editorial advisors over the years, I feel well prepared to take the reins.

Speaking of learning on the job, I'd like to introduce you to our new associate technical editor, Sarah Stefancin. Based outside Cleveland, OH, she is a 2021 graduate of the University of Mount Union who worked as a toxicology report writer for Frontage Laboratories before joining us. She's as new to bioprocessing as I was when I jumped into this pool at her age nearly 30 years ago, and I recognize much of myself in Sarah's confident and curious approach to our subject matter. And she is the first BPI editor not to be based here in Oregon — as we move with the times in this post-COVID reality.

If you're involved on the production side of our Ask the Expert webinars, you already may have met Sarah virtually because she took over the reporting aspect of that series quite handily and almost immediately upon joining our crew. That gives our more established associate editor,

Josh Abbott, the room he needs to take ownership of our focus-on articles — which in turn allows managing editor Brian Gazaille and me to focus much of our attention on the technical side of things. We hope that this reshuffling of editorial work will help things go smoothly in the future. And it should give our authors and advisors a good idea of who to contact for what, something that hasn't always been so obvious. . . .

And there I go again, talking about processes. Well, this is *BioProcess International,* after all! Standard operating procedures, product quality attributes, process optimization, and the like make up our bread and butter around here — and yours too. We've always made the point that the *Process* in our name refers to the *entire* process of biopharmaceutical development: not just manufacturing, but also product characterization, developability assessment, formulation, business and project management, outsourcing, regulatory affairs, hiring and training, analysis and quality control, supply chain and logistics, and so on. From the outside, it may seem like pretty narrow coverage — but we know (because we've learned it from you) that the breadth of topics is surprisingly wide within our niche.

Managing the publication is a little bit like that. Many readers are surprised to find that the editors are also designers — making figures and tables and layouts and such — in addition to being interviewers, writers, and hands-on copyeditors. We all seek out expert contributions (the "meat of the magazine") and commission papers from freelancers as well. We all travel and meet with authors and readers and advertising clients — and we all work on custom projects for our supplier friends too. By necessity, we work closely with our sales representatives, production crew, webmasters, and publisher to plan coverage and schedules. We enjoy our colleagues and can't imagine doing things any other way.

So welcome to 2024. It's an election year in the United States of America, and we expect the usual political craziness to multiply if not exponentially then at least factorially. (For example, it's rumored that campaign spending will far exceed the billions of dollars that circulated in 2020. And the events of 6 January 2021 weigh heavy on our minds even now.) Your industry is likely to take a few financial and public-relations hits along the way, and there's no telling what kind of unforeseen events could derail operations around the globe. Weather, warfare, and other world events can upset even the best-laid business plans, as we've all learned in recent years. It's best to cultivate flexibility and rapid-response capability — and not to ignore the red flags when they appear.

The authors in this issue and featured report offer several approaches to help you build such a mindset, whether it's future-proofing your workforce, setting people up for success, or planning now for changes ahead. On the technical side, we're scaling up and managing risk, ensuring product quality through environmental monitoring, analytics, and process engineering. So let's get into the weeds together — but keep that big picture in mind.

BioProcess Insider

The BioProcess Insider *portal delivers financial and business news online alongside expert views about the commercialization of biopharmaceuticals. Here are a few recent stories edited for print. Visit* https://bioprocessintl.com/category/bioprocess-insider *to find in-depth discussion and sign up for the thrice-weekly newsletter.*

Will 2024 Reach an Inflection Point in Bioprocessing?

by Dan Stanton

After two years of unprecedented demand driven by COVID-19–related vaccines and products, bioprocessing vendors began to report falling orders in the latter half of 2022. This destocking dynamic was explained and accepted as a "normalization of demand" that led to a 15% to 20% drop in quarterly sales year on year among many of the largest biotechnology companies.

Although some experts earmarked 2023 for a turnaround, market conditions have recovered slowly. Companies are continuing to work through the inventory that they had stockpiled during the pandemic while also conserving capital because of financial pressures. The sector was further rocked by macroeconomic factors such as inflation and political unrest. But speaking at the JP Morgan Healthcare Conference on 8–11 January 2024, two of the industry's largest suppliers suggested that 2024 will be a year of recovery.

Leaders at Danaher Corporation, which absorbed Pall Life Sciences in May 2023, had hoped that Q4 2023 would be a turnaround point for the industry. Danaher chief executive officer (CEO) Rainer Blair said that although sales were indeed better in Q4 than they were in Q3, that result was not unexpected. "Q3 tends to be the lowest quarter of the year in terms of its activity levels," he said. "In the fourth quarter, we continued to have constructive dialogue with our customers [and have] more anecdotes about customers [returning to their] regular ordering patterns." However, "many of these constructive dialogues have yet to show up in data points of regular order submissions. We would say that we are not yet at the inflection point." He added, "We didn't achieve the original financial goals that we set out to, and we'll look forward to getting back to that track record in 2024."

Marc Casper, CEO of Thermo Fisher Scientific, said that the diminished growth in 2023 was worse than predicted a year ago. "The year wound up [being] more challenging, even in the moderation of growth that we had reflected upon," he told JPM delegates. He cited a difficult macroenvironment and a declining Chinese economy as reasons for that decline, adding that Thermo Fisher's bioproduction business – which includes cell culture media, single use technologies, and purification platforms – has historically grown 15% to 20% year on year, and such double-digit growth will return in due course. "The future is very bright in bioproduction," he said.

MassBay Community College Bulks Out Biotech Training Centers

by Sara Healy

In November 2023, the Commonwealth of Massachusetts and Massachusetts Life Sciences Center (MLSC) provided grant funding of more than US$1.25 million to Massachusetts Bay Community College (MassBay) in Wellesley. The funding will help MassBay acquire equipment, including bioreactors, fermentors, and advanced systems for digital polymerase chain reaction (dPCR) chromatography, tangential-flow filtration (TFF), flow cytometry, and fluorescence-activated cell sorting (FACS. The MassBay Center for Biomanufacturing Education and Workforce Training (CBEWT) will join the Center for Therapeutics and Genomics Training (CTGT), which has trained biomanufacturing technicians since 2020.

MassBay president David Podell said, "We are extremely grateful for the continued support of the Commonwealth and MLSC to ensure our science, technology, engineering, and mathematics (STEM) students have access to a lab with state-of-the-art equipment that will provide them with an experience they can't receive anywhere else." He added, "Biotechnology and biomanufacturing are growth sectors in our regional economy, and our students are prepared for the modern workforce. The opening of the centers is key to providing a well-trained pipeline of future employees that will build on Massachusetts's well-known strength in STEM-based industries."

The Boston area hosts facilities for many of the largest pharmaceutical companies in the world and employs a sizeable biotechnology workforce. The percentage of employees specialized in one or more STEM areas in Massachusetts is 21%, well above the 14% national average.

Nirmal Singh, director of the newly opened centers at MassBay, recognized how the demand for skilled technicians has intensified in the past few years. "Our biotech programs are crucial [for] filling the employment gap and fostering regional economic development," he said. "Not only do we prepare [students] for successful careers, but we also shape a new generation of highly skilled

biomanufacturing technicians. Together, these centers serve as a launch pad for our students, propelling them toward impactful careers that contribute to a more equitable and sustainable bioeconomy."

MassBay will partner with area high schools to offer students access to the college's biotechnology programs, which include new courses in genomics, cell and gene therapies (CGTs), and biomanufacturing.

ADCs Flying High Amid Surge in M&A, Licensing, and Investment

by Dan Stanton

Antibody–drug conjugates (ADCs) work by attaching a cytotoxic small-molecule drug to an antibody that is raised against a specific molecular receptor, in principle creating a highly targeted and effective therapy. Pfizer's Mylotarg (gemtuzumab ozogamicin) was the first such drug to receive US Food and Drug Administration (FDA) approval in 2000, but since then, approvals have been relatively sparse. As of August 2023, only 13 ADC products have reached the market.

ADCs have been slow to reach patients for a number of reasons. Complications often arise during development, such as with the linker, the location and amount of payload to be conjugated, and the prevalence of unexpected toxicities and resistances to therapies that researchers discover during trials. But despite such difficulties, interest in ADCs from large pharmaceutical and service companies remained high throughout 2023.

Licensing: In November 2023, Bristol Myers Squibb (BMS) signed a US$100 million licensing deal with South Korea's Orum Therapeutics, gaining access to its ORM-6151 program: an anti-CD33 antibody-enabled degrader to treat acute myeloid leukemia scheduled for phase 1 clinical trials. The molecule uses conjugation technology provided by Orum's GSPT1 degrader-based platform and adds to BMS's ADC pipeline. In April 2023, the company inked a potential $1 billion deal to develop ADCs with German developer Tubulis.

Other large pharmaceutical companies have followed similar ADC strategies in 2023. Merck & Co. paid $4 billion to codevelop and market three ADCs with Daiichi Sankyo in October, and GSK paid $85 million for the global rights to a gynecology-cancer ADC from Hansoh Pharmaceutical. Germany's BioNTech entered the space in April through a $170 million deal with Duality Biologics. And on the technology front, Astellas Pharma teamed with Sony Corporation to access Kiravia Backbone polymeric material as a linker to improve therapeutic efficacy of its ADC candidates.

Mergers and Acquisitions: Pfizer acquired Seagen in a $43 billion deal in 2023, the largest pharmaceutical merger since 2019. That boosted Pfizer's portfolio with approved ADC cancer products Adcetris (brentuximab vedotin), Padcev (enfortumab vedotin), and Tivdak (tisotumab vedotin). It also will add about 10 (mostly ADC) candidates to its developmental pipeline. Meanwhile, Eli Lilly agreed to buy preclinical ADC company Mablink Bioscience.

CDMO Investment: In 2023, a flurry of activity from contract development and manufacturing organizations (CDMOs) broadened their capabilities to support the ADC space. Samsung Biologics has invested heavily in ADCs, introducing new capabilities to its site in Songdo, South Korea, and investing in ADC entities through its Samsung Venture Investment Corporation wing. Fellow South Korean CDMO Lotte has followed suit, bringing ADC services to a converted former BMS animal health laboratory at its US site in East Syracuse, NY.

WuXi Biologics and WuXi AppTec, meanwhile, have collaborated on a joint venture called WuXiXDC to focus on bioconjugation. The new venture is conducting a $500 million initial public offering (IPO) in Hong Kong, China.

Lonza has been part of the ADC sector since 2006 and boasts involvement in many approved products. In the past year, the CDMO has ramped up its ADC and conjugation-related investments, acquiring technology from AbTis and Simris and paying over $100 million to acquire Dutch company Synaffix.

A New Era of ADCs: ADCs have been around for over two decades, and according to some studies, more than 100 such products are currently undergoing human studies. Company pipelines reflect the current surge in activity, but the question remains: Why now?

"Development of ADCs has accelerated recently because research over the past decade has resulted in a substantial expansion of available options for antibody design, type of linker, and the active payload," said Janice Reichert (chief operating officer (COO) of nonprofit organization The Antibody Society) in a September 2023 article for *BioProcess Insider*. "The number of validated target antigens has also substantially increased."

Conjugation technology has significantly advanced since the first generation of ADCs hit the market in 2000. "Most ADCs to date have been made by nonspecific conjugation of drug to antibody at multiple potential sites," says Geoff Hale, CEO of antibody technology at mAbsolve. "This leads to heterogeneity and complexity for manufacturing and quality control. More recently, ADCs are being developed with site-specific modification, so [they] are more homogenous and reproducible. I expect this trend to increase in [the] future."

Women-Focused Networking: BPI Hosts the CGT Circle's 10th Event

by Millie Nelson

In November 2023, *BioProcess International* hosted a CGT Circle networking event in London, UK, to gather and celebrate like-minded women in a professional and personal environment. The *CGT Circle* is a networking platform designed to empower and connect women who contribute to the CGT sector.

The three founders of the CGT Circle — Kate Fynes, a chemistry, manufacturing, and controls (CMC) translation consultant at eXmoor Pharma; Nicola Ambler, director of Olmec Connections; and Lindsey Clarke, vice president of commercial operations at MicrofluidX — said that the demand for community-led networking steered them to launch the platform, which aims to create "decentralized networking" for women in life sciences. In turn, the platform enables women at different career stages to share experiences and support one another professionally and personally.

Members of the CGT Circle gathered at Informa's London offices for networking and festivities. Alongside holding conversations that spanned both professional and personal lives, women decorated holiday baubles and participated in other ice-breaking activities.

Taylor Beavis, marketing manager at Astrea Bioseparations, said the event "was a truly enriching experience that seamlessly blended professional insights with festive holiday cheer. Being able to engage in discussions about the latest advancements in [CGTs], share experiences, and forge connections with like-minded professionals was both empowering and enlightening. As a woman in the industry, being a member of this group is invaluable. Such events not only broaden my professional network, but also serve as a source of inspiration, reinforcing the importance of diversity and inclusion in driving innovation within the [CGT] landscape."

"I thought it was a great event, and it was lovely to meet, like-minded individuals and catch up with some familiar faces [from] previous CGT Circle events," said Nikita Patel, senior scientist at INmune Bio. "It feels good to be part of a community that is so friendly, encouraging, and approachable, where we can share our experiences and learn from each other."

Charlotte Deacon-Wild, senior scientist at ViroCell Biologics, added, "My main reason for attending the event was that my family and I have just moved to London, so it was an opportunity to reach out to new people."

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ATMPs and Traditional Biologics Face Similar Challenges

by Millie Nelson

The complex nature of advanced therapy medicinal products (ATMPs) is a frequent discussion topic at life-science events around the world. Delegates often discuss high manufacturing costs, new innovative payment models, patient access worries related to different healthcare systems, and difficult regulatory pathways to approvals. However, at the bioProcess UK event in November 2023, a panel debated whether the challenges facing ATMPs are any different from those that faced protein biologic developers some 20 years ago.

Steve Bates, CEO of the UK Bioindustry Association (BIA), channeled his inner Fiona Bruce by opening the panel in the format of the popular BBC television program *Question Time*. Each panelist represented a "political party" and answered questions from the audience on behalf of their respective parties. One audience member asked, "Does the panel think that [the] issues ATMP companies are facing are different to what traditional modality companies faced?"

Representing the "CGT Party," Ian McCubbin, chair of RoslinCT and the CGT Catapult, said that "there are a huge number of similarities [between] biologics and new modalities. [However], I will repeat that ATMPs are curative, and [most other] biologics are not. The issue with CGTs is that they are expensive. But we have seen dramatic changes in manufacturing technology to bring costs down."

Steve Bagshaw, chair at High Value Manufacturing Catapult and representative of the "Complex Medicines Party," cited patient access issues associated with ATMPs and told the audience, "Biologics were giving people hope, whereas ATMPs are giving certain people in certain areas hope." He said that the industry "will have a multimodality approach" and that people "will need biologics and ATMPs."

McCubbin countered Bagshaw's claims about the limitations of ATMPs. He cited the recent UK approval for the world's first therapy based on clustered regularly interspaced short palindromic repeats (CRISPR) gene editing: Casgevy (exagamglogene autotemcel [exa-cel]) from Vertex Pharmaceuticals and CRISPR Pharmaceuticals. According to McCubbin, the product is likely to treat "hundreds of thousands of patients over the next 10 years."

From the "Investor Party," Alex Hamilton, partner at Syncona, spoke about the switch in skillsets from chemistry to biology taking place within the biopharmaceutical industry. He said that the transition is probably easier now than it has been historically but that the industry still has challenges to overcome. He said that when working with ATMPs, companies must think about how many doses they can produce from each batch and how they can do so consistently.

Cath Green, head of Clinical Biomanufacturing Facility and partner at Vax-Hub Global, University of Oxford, represented the "Academic Discovery Party" and discussed the ongoing talent shortage within the industry and the realization that now companies need employees who have digital skillsets. "We need more data scientists now." To address this issue, Green suggested that people think about the young scientists who will drive innovation in the future.

Bayh-Dole Act Nurtures Innovation, Says Nobel Prize Winner by Gareth Macdonald

The Bayh-Dole Act of 1980 gave universities, nonprofit research institutions, and small businesses in the United States the right to own, patent, and commercialize inventions that they develop under federally funded research programs. The act also prompted a 40-year debate over appropriate incentives for innovation and the scope of government authority. The pandemic intensified such discourse, with some people calling on the government to use its so-called "march-in" rights to quell concerns about companies monopolizing patents and setting exorbitant prices for their products.

Innovation: The US Congress passed the Bayh-Dole Act to incentivize industry and academia to work collaboratively, said advocate Joe Allen, who served as a staff member on the US Senate Judiciary Committee with former Senator Birch Bayh. "If a university or a small company makes an invention with government funding, they own it. They can then license it for commercialization." He added, "The biotech industry came out of Bayh-Dole. The early stage inventions were made at [the University of California, Berkeley] and Stanford [University]; not a single big drug company was interested. So they started spinning off small companies, which is still how the biotechnology industry works."

About half of new drugs approved in the United States are discovered/developed by small companies. And according to Allen, such numbers prove the merits of the Bayh-Dole Act. "Bayh-Dole has been a tremendous success. We've become a leader in life sciences." Numerous projects have benefitted from Bayh-Dole, including the work on nucleoside modification and messenger RNA (mRNA) immunogenicity that underpinned COVID-19 vaccines and that earned Katalin Karikó and Drew Weissman the Nobel Prize for Medicine in 2023.

Nurturing: The act also aided the development of a human immunodeficiency virus (HIV) therapeutic by a team led by Dennis Liotta at Emory University in the 1990s. Liotta said, "After we did the HIV work, a colleague and I started a company called Pharmasset. Out of that company came sofosbuvir, which was the breakthrough drug for hepatitis C." He said that his team developed molnupiravir, which was granted emergency-use authorization by the European Medicines Agency (EMA) to treat COVID-19. "We couldn't have even conceived of doing these things without . . . the Bayh-Dole Act."

Benefits from the legislation continue to shape drug research according to Karikó, who cites the environment that the act creates for current researchers as a major advantage for the US sector. She compared the research climates in Europe and the United States and praised the resources available to American students and researchers provided by the Bayh-Dole Act. "That environment is very nurturing," she said.

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Dan Stanton *is founding editor,* ***Millie Nelson*** *is editor, and* ***Sara Healy*** *is a reporter, all at* BioProcess Insider*; dan.stanton@informa.com.* ***Gareth Macdonald*** *is a freelancer contributor.*

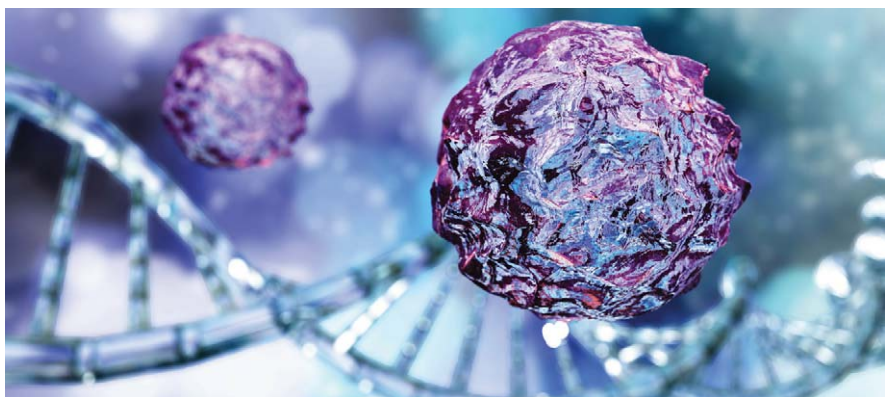
Getting Cell Therapy Right at the Source

Becky Cap

The materials used in cell and gene therapies (CGTs) come from human donors, introducing variability that complicates manufacturing. For consistency in product safety and efficacy, it is critical to address donor variability and optimize manufacturing at every step of CGT production. And that starts before cell collection.

Despite its rapid growth, the CGT sector has yet to coalesce robust, evidence-based standards in areas such as cell collection and donor screening. That is partly because many new technologies are in development in addition to already-approved materials such as chimeric antigen receptor (CAR)–T and pancreatic islet cells as well as enabling platforms such as clustered regularly interspaced short palindromic repeats (CRISPR) and viral vectors. To further complicate matters, donor-eligibility regulations differ by country, and there is bifurcation between requirements for autologous and allogeneic therapies (**1**).

The result is that developers — especially in early stages — may struggle to qualify their starting materials, leading them to develop narrow criteria that increase time and cost, leaving them unprepared to recruit donors. By understanding better how blood centers screen donors and collect and test cells, companies can optimize starting materials and accelerate their clinical use.



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> The most important distinction is between autologous and allogeneic therapies, for which the **APHERESIS** processes differ greatly.

Automated apheresis enables efficient isolation of blood-derived starting material for advanced therapies, but it remains a time-consuming process with many variables that can affect a final leukopak product. The technology involves

- removing a specified volume of blood from a donor
- moving collected blood through a closed system with anticlotting agents
- separating components by centrifugation and/or filtration
- collecting target components in a leukopak
- returning the blood volume to the donor.

Developers must understand their starting-material needs when planning a donor screen, including biological and social screening criteria, preferred apheresis endpoint, target cell type and required volume, planned use of materials, and preferred shipping conditions. Perhaps the most important distinction is between autologous and allogeneic therapies, for which the apheresis processes differ greatly.

Getting the Most from Patient-Derived Blood

Apheresis is simpler in many ways for autologous products than for allogeneic products. Although baseline infectious-disease screenings are conducted in the same way in both processes, the resulting data are used differently. Because patients receive their own modified cells in autologous therapy, the presence of certain infectious pathogens does not preclude those cells from use. Instead, test results of starting materials and final products are compared postmanufacturing to ensure that the process has not been contaminated.

A patient's apheresis experience will differ depending on developer

specifications. In some cases, blood collection ends upon reaching a target count of the required cell type, which is typically highest for clinical-grade good manufacturing practice (GMP) materials. In other cases, blood centers might use different procedures, such as limiting the time that a patient spends being apheresed or using a predetermined number of filtrations for a certain blood volume. Testing for predonation leukocyte count and total blood volume helps blood centers determine how long collection will take.

Leukopaks can be filtered for specific cells on site. Depending on a developer's need, collection can be broad enough to include all peripheral blood mononuclear cells (PBMCs) or narrow enough to include only T-cell subsets that express specific markers such as CD4 or CD8.

Apheresis sessions take several hours. Before the process, a blood center will either test a patient's CD34+ cell count or perform a complete blood count with differential (CBCD) to assess readiness for the procedure and estimate the amount of blood needed to achieve a desired target. During apheresis, the blood center will perform a cell count from the leukopak itself to calculate the time needed to meet a target. An apheresis technician must monitor start and end times to keep from prolonging the procedure, which carries risk for both patients and collection processes. If collection does not meet target counts, the patient may need to return for an additional session.

Once a developer's target is met, the apheresis site performs finished-product testing using, for instance, flow cytometry or CBCD. Results of a CBCD can help determine the quantity of cells and assess cell-type proportions. The goal is to ensure that the cells of interest have been collected with minimal contaminating cells, such as red blood cells and granulocytes. Additional quality control (QC) testing, such as for cell viability or sterility, is performed before shipping or processing to capture the percentage of viable cells in a leukopak.

Leukopaks can be shipped either fresh or frozen before manufacturing. Distance from collection to manufacturing site, travel logistics, and developer protocols dictate whether fresh shipments can be supported. Fresh leukopaks are shipped on the day of apheresis collection and typically are processed immediately upon receipt. Alternatively, leukopaks can be frozen ahead of manufacturing, and cryopreservation can occur either at the collection site or at a separate location.

When they are not manufactured fresh, leukopaks should be frozen within four to six hours of collection. A cryoprotectant such as dimethyl sulfoxide (DMSO) is added before leukopaks are cooled in a controlled-rate freezer to minimize cell death. The product is then shipped in a temperature-controlled container with a data logger that enables workers to monitor temperatures throughout the shipping process.

Allogeneic Adaptations

Allogeneic apheresis differs in part because the blood used during the process comes from healthy donors, which significantly reduces some variability observed in material from patients, who may be at advanced stages of disease. But for allogeneic therapies, developers are trying to understand the connection between baseline donor conditions and finished product quality. Thus, screening criteria are built on assumptions, especially regarding donor age and lifestyle.

Allogeneic apheresis products start with a CBCD. Unlike autologous products, almost all allogeneic leukopaks are cryopreserved for shipping to a manufacturing site, where they can be processed into material used to treat 100 or more patients with as many therapeutic doses. But although healthy donors can spend more time in the apheresis chair without health concerns, allogeneic therapies have their own complications.

When allogeneic therapies are intended for use in multiple jurisdictions, donor screening needs to comply with regulations for each geography. For example, Japanese regulators require that donors test negative for Epstein–Barr virus (EBV), but 90% of the world's population has been exposed to that pathogen, complicating recruitment (**2–4**). A trial with an arm in Tokyo might need to apply such requirements across its entire clinical network. Different countries often have distinct rules about product pooling, a situation that can necessitate additional testing and donor categorization depending on the presence of protein markers such as human leukocyte antigens (HLAs).

Within the autologous therapy space, there is a shift toward DECENTRALIZED MANUFACTURING that may change how leukopaks are managed postcollection.

Therapeutic developers often seek criteria that are rare among potential donors, and the pathway from a prospective donor to a collected leukopak requires careful management. That can include extensive screening and associated costs, necessitating that researchers balance the desire for specific donor characteristics against program-success criteria — particularly when considering the long-term needs for starting-material scale-up.

Another consideration is donor recallability. The field remains uncertain about some of the factors that determine the efficacy of certain donors' cells. Developers may want to recruit "super donors" who can give PBMCs for multiple production runs. Donor characterization for cells of interest using flow cytometry can help identify super donors who can provide starting material that is especially well suited for therapeutic development. Once such donors have been identified, developers can ask them to return for additional collections. A well-characterized donor registry facilitates targeted recruitment, but large-scale donor screening could be necessary to identify super donors.

continued on ***page 21***

Top Trends in Global Biomanufacturing

20 Years of Analyzing Biologics Production and Supply

Erica L. Friedman

The biopharmaceutical industry continues to grow globally as it recovers from the COVID-19 pandemic. The significant COVID-driven expansion of bioprocessing activities worldwide created supply-chain pressures, availability problems, and delivery delays. Suppliers increased revenues by reducing standard discounts, and contract manufacturers overordered equipment and consumables to reduce their supply-chain risks.

According to the BioPlan Associates *20th Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production*, industry growth is stabilizing, shifting back to previous standards of 12–13% annually (**1**). However, many subsegments in bioprocessing — e.g., that for single-use (SU) devices — continue to experience post-COVID downturns in growth.

Over the past 20 years, the biomanufacturing industry has shown great adaptability to economic swings and supply-chain challenges. Healthcare tends to be recession resistant, and the biopharmaceutical industry's resilience comes in part from strong innovation pipelines, advances in novel modalities such as cell and gene therapies (CGTs), and demand for biosimilars in emerging markets. Technological improvements made by bioprocess suppliers also are helping to streamline production and enable cost-effective bioprocessing globally. Below are some key trends identified in BioPlan's report.

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Top Trends in 2023

Supplier revenue growth rates almost doubled during the COVID-19 pandemic. In 2023, the average biopharmaceutical-industry supplier reported a return to normal revenue trends (Figure 1). However, because survey respondents tended to represent companies much smaller than most of the industry's primary suppliers, the unweighted "average" revenue change better reflects the experiences of smaller companies. Suppliers estimated overall growth in bioprocess-related sales by a rate of 13.1% in 2023, down from 24.5% in 2022. The industry is adjusting to the end of extraordinary growth during the COVID period and has returned to an annual growth rate that aligns with trends from the past 16 years.

COVID-related supply-chain worries have yet to abate. We asked vendors how the COVID-19 pandemic was affecting their businesses. The most common response was that they (not unexpectedly) were spending more time than ever addressing customers' supply-chain concerns. In 2023, 85.5% of suppliers reported having that experience. In effect, the pandemic has created a long-term drag on industry suppliers. Amid increases in both demand and supply-chain problems, manufacturers' fears about unavailability of supplies are influencing the entire biopharmaceutical pipeline. That problem is expected to continue causing disruptions, at least into the coming year.

Respondents identified 14 key areas for development of new upstream bioprocess products and technologies. In 2023, the top three areas related to upstream production were cell-culture media, bioreactors (especially in SU formats), and information technology (IT), including automation, software, and networking. The number of respondents interested in IT solutions increased from 21.1% in 2022 to 29% in 2023.

Demand continues to increase for low-cost SU devices. Over the past 15 years and especially for clinical-scale

operations, SU bioprocessing devices such as bags, tubing, bioreactors, and mixers have taken a leading position over stainless-steel formats for biomanufacturing equipment. When COVID-19 created serious availability problems, cost concerns dissipated. Now, as the pandemic abates, the industry is returning its focus to cost. Respondents identified lower-cost SU devices as the most important area of interest for new products and technologies in 2023.

Cost per gram for recombinant proteins continues to decline. The overall cost to manufacture recombinant proteins has decreased steadily for the past five years. BioPlan has observed a 29.5% decrease over four years in total cost per gram of antibody — partly the result of continuing improvements in process efficiency and productivity.

Five-year capacity constraints are predicted to decrease despite COVID-19. In 2023, only 17.2% of respondents expected "severe" or "significant" capacity constraints within five years. That figure is down from 21.7% in 2022 and continuing along a downward trend. The number of companies expecting no capacity constraints over that period remains about the same as last year.

Automation is needed to minimize capacity constraints. Respondents identified 27 ways in which they might prevent capacity constraints. Among the most important solutions selected was to add automation capabilities, as indicated by 34.5% of respondents.

Biomanufacturers and suppliers have been unable to hire and retain both new and experienced production staff. Survey respondents identified several major factors that they expected would constrain their facilities' biologics production capacity over the next five years. Of the 22 factors evaluated, the most critical staffing factor involved finding both new and experienced technical and production personnel (Figure 2). Continued growth in capacity demand at both new and existing biomanufacturing facilities is impeding efforts to train and retain manufacturing staff.

Gene-therapy manufacturers critically need better analytical methods and instrumentation. Among the 11 factors being evaluated, gene-therapy companies identified virus/vector analytical testing as needing the most improvement. That factor also showed the most dramatic increase in importance, with 46.7% of respondents indicating need in 2023 compared with 22.7% in 2019.

Applications of disposable equipment proliferated significantly between 2006 and 2023. Disposables uptake has grown relatively steadily in nearly all areas of biopharmaceutical manufacturing and for a breadth of applications. This year, more than half of the 16 applications measured showed moderate or large increases in use. "Bags, empty" took the top position again this year, with 93.9% of respondents indicating their use of such disposables (up from 64.5% 17 years ago in 2006). Steady growth in the use of such devices also reflects increases in SU-device uptake more generally.

The United States of America and Germany are today's top international outsourcing destinations. We inquired about five-year plans for international expansion in biomanufacturing capacity. Of the 36 countries identified as potential foreign (outside a respondent's country) outsourcing destinations, respondents most often highlighted the United States, with 47.8% of non-US respondents indicating a "strong likelihood" or "likelihood" that they would outsource production to US-based facilities.

Since we started recording data for possible international outsourcing, however, we have observed dramatically increasing interest in China as a potential outsourcing destination.

Outsourcing has become commonplace over the past 17 years of biologics production. The percentage of the industry that contracts for manufacturing services is clearly on the

Figure 1: Growth in vendor sales for biologics from 2007 through 2023, as represented by an annual average growth rate with a two-period moving trend line; unweighted data come from reference (**1**).

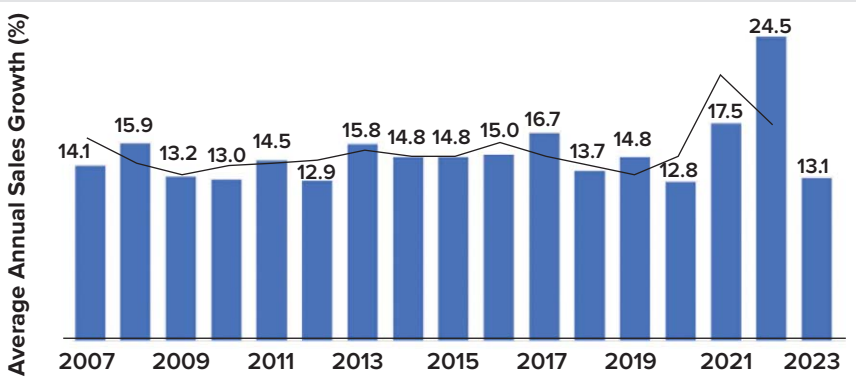


Figure 2: Percentage of BioPlan survey respondents who cited the "inability to hire new and experienced technical and production staff" as a likely constraint on biomanufacturing capacity over the next five years (**1**)

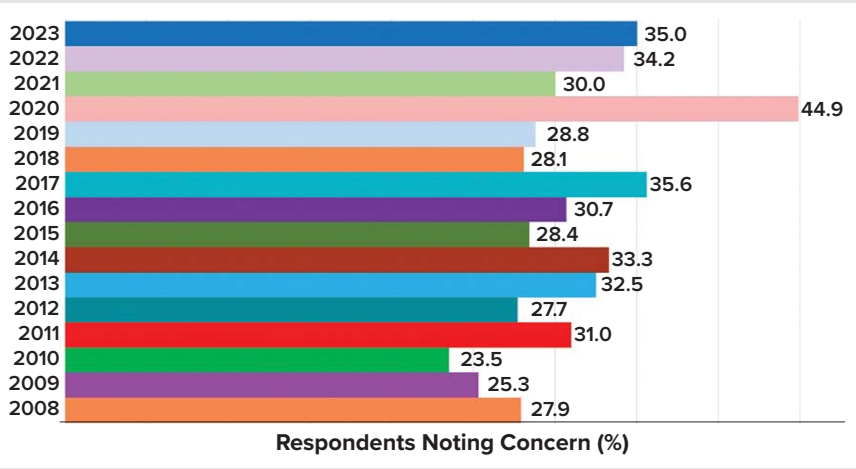
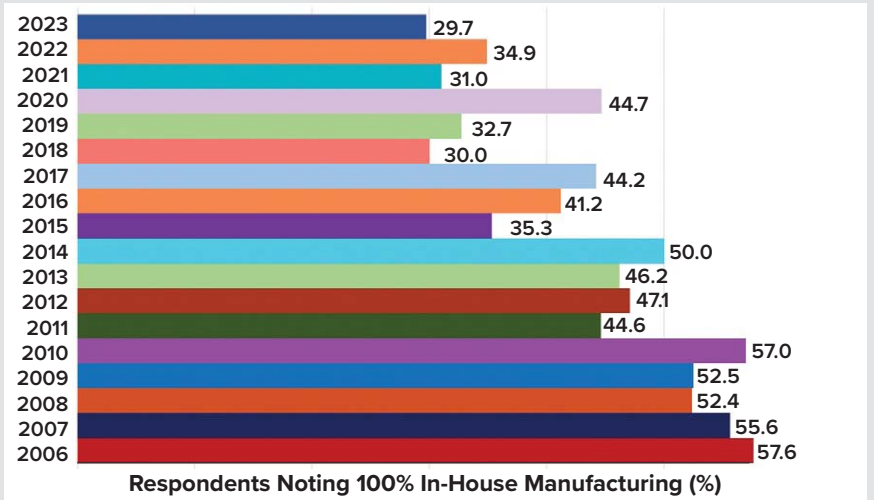


Figure 3: Percentage of respondent companies that reported performing all manufacturing activities internally, by year (**1**)



rise as fewer drug developers seek to keep their entire production processes in house. That is the case particularly for developers whose products require mammalian-cell culture, with 70.3% of such respondents projecting at least some outsourcing in 2023 (Figure 3).

Biomanufacturing offshoring is likely to increase in low-cost regions over the next five years. We evaluated responses from 2011 through 2023 regarding biomanufacturers' plans to outsource (internationally) at least some of their biomanufacturing operations to low-cost regions. This year, 67.8% of respondents reported that that they would offshore at least some of their biomanufacturing operations to such locations within the next five years. That percentage is up significantly from pre-COVID responses. Consider, too, that 12 years ago (in 2011), only 29.6% of companies believed that they would offshore work to lower-cost regions within five years.

Suppliers and technology developers are working to improve bioprocess automation. Automation is the hottest area of opportunity for vendors' new-product–development departments. Automation will be critical if the biopharmaceutical industry is to continue maturing and optimizing. We asked suppliers what top new technologies or new-product–development areas their companies were working on. They highlighted 60 areas for work and investment, many relating to improving efficiency. The largest percentage of responses from vendors (of all types) identified automation.

Two-thirds of clinical bioproduction processes leverage SU components. Disposable bioprocess equipment continues to gain industry acceptance because of its advantages for both contract manufacturing organizations (CMOs) and drug developers. Across the board, we observed increased application of SU systems at all production scales, from research and development (R&D) to commercial manufacturing and for both up- and downstream operations. Most notable is that SU equipment was applied in 64.0% of upstream clinical production processes, up from 45.0% in 2021.

Highlighted Developments

Revenue Growth During COVID: Before the COVID-19 pandemic, the biomanufacturing-supply industry grew at an annual rate of ~13–14% for decades. During the pandemic, year-on-year revenues for the average supplier increased by nearly 25%. That near-doubling in growth was experienced fairly broadly across the industry. Now, however, many biomanufacturing suppliers have experienced significant downturns in revenue. In some subsegments, such as that for SU equipment, companies continue to report 20–30% reductions in revenue.

As described above, our annual report points to a reversal of that trend and a return to the more standard growth rate of 12–13%. Note, however, the unweighted "average" revenue change is more reflective of smaller respondents' experiences than of large suppliers' experiences. The latter companies made big gains — but then had large downturns.

For the SU and related subsegments, a return to normal growth has yet to occur. When performing research for other reports, we found that most SU suppliers this year have experienced a 21–24% decrease in order volumes. However, such companies also expect a return to normal growth by mid-2024.

The rapid downturn in revenue and order volumes during 2023 has been felt most by segments that experienced rapid increases in revenue. Several factors are implicated in the decline. Manufacturers have begun drawing down safety stocks and inventories that were overordered during the pandemic. Suppliers have returned to standard discounting, a strategy that accounts for some drop in revenue. And new competitors entered the market during the pandemic. Meanwhile, customers are "belt-tightening" in response to economic (e.g., inflation) and political concerns. End users also continue to harbor concerns about supply-chain risks leading to dips in production capacity growth.

However, several factors are driving underlying growth in biomanufacturing operations and supply. New biopharmaceutical companies are developing and/or expanding bioprocessing capacity. New biological products continue to enter clinical trials, and drug-development pipelines remain full. Because numbers of new-product approvals continue to increase, more products are being manufactured. Marketed drugs also are being approved for new indications, leading to a higher volume of products being manufactured. At the same time, aging populations in major-market countries are driving demand for biopharmaceuticals, and the middle class continues to grow in many industrially developing countries. Supporting all of those factors is the ability to manufacture biologics at scales large enough for global populations.

Inability To Hire and Retain Experienced Staff: Manufacturers' inability to hire qualified operational and manufacturing staff has created production constraints. As it has for the past 15 years, this slow-burning problem continued to expand in 2023. The most

Continued on ***page 36***

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Comparability for Cell and Gene Therapy Products

A Challenge and an Opportunity

K.R. Poudel, Zenobia Taraporewala, Diane Blumenthal, Deep Shah, and Kathy Francissen

Cell and gene therapies (CGTs) are the fastest growing segment of biotechnology products, with chimeric antigen receptor (CAR) T-cell products and gene therapies using adenoassociated virus (AAV) vectors dominating the landscape. To meet the increasing demands of these promising therapeutics, manufacturing changes often are implemented during clinical development as sponsors scale production up or out and optimize processes for productivity and performance. A well-designed comparability study is critical for demonstrating an absence of adverse effects on product quality, safety, and efficacy when a manufacturing change is made. The complexity of CGT product modalities and their current level of characterization pose challenges to the approaches typically used for assessing comparability, as guided by the principles described in ICH Q5E (**1**).

Comparability strategies that are tailored to each program and product type are key to successfully implementing manufacturing changes throughout product development and commercialization. Such adjustments can help streamline development, reduce cost of goods sold (CoGS), and ultimately increase access for a wider patient pool. Implementing changes to meet the demands of clinical supply and commercialization has presented distinct obstacles for CGT products. For example, transition from an adherent to a suspension-adapted cell culture process for manufacturing AAV vector-based products can increase output while increasing the level of process- and product-related impurities in final products.

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For cell-based products, the inherent variability of cellular starting material introduces variability that can persist into a final product. That can make it difficult to distinguish the manufacturing process from the cellular starting material as the source of differences in final product quality. Many cell-based products are made to order for single patients (autologous products). In such cases, limited material availability for analytical evaluation creates constraints on comparability study designs. Similar issues must be addressed in planning comparability studies for viral-vector–based gene therapy products in development for rare diseases.

Overall, the complex manufacturing processes for CGT products combined with currently limited understanding of clinically relevant product quality attributes (PQAs) make it important to design a "fit for purpose" comparability approach. Developers can benefit from discussions at forums such as CASSS meetings for sharing of knowledge, novel technologies, experience, and case studies that can improve understanding of how guidance from regulatory agencies can be applied in managing manufacturing changes and expediting the delivery of life-saving products to patients. In its inaugural one-day Cell and Gene Therapy Products (CGTP) summit held 26 June 2023, CASSS brought together experts from industry, academia, and regulatory agencies to discuss challenges and opportunities in the design and implementation of comparability strategies for CGT products. This summit was held just before the US Food and Drug Administration's (FDA's) release of a draft comparability guidance in July 2023 (**2**).

The morning session of this summit was devoted to viral-vector–based gene therapies; the afternoon session was focused on cell-based gene therapies. Each talk in the morning session was

followed by a panel discussion in which experts fielded questions from online and onsite audiences. The presentations and the panel discussions focused on comparability studies for AAV-based gene-therapy products and CAR T-cell therapies, with related statistical considerations for study design. Learnings from the first developed AAV-based and CAR T-cell products can be applied to other product types in the CGT family that will be advancing rapidly toward commercialization in the near future.

In an introduction to the summit, the chairperson noted that the overarching principles of comparability assessments described in the ICH Q5E guidance should be applied to CGT products using a risk-based approach. Notably, comparability does not necessarily mean that the quality attributes of prechange and postchange material will be identical, but rather that they are highly similar and that the existing knowledge is sufficiently predictive to ensure that any differences will have no adverse effects on safety or efficacy. When considering a manufacturing change, a product developer should consider its potential influence on other parts of the process and on product quality — and should assess the potential risk of adversely affecting product safety or efficacy.

The following points also were highlighted: Determination of comparability can be based on a combination of analytical testing, biological assays, and in some cases, nonclinical and clinical data. Comparability assessments rely on a body of knowledge, and product and process understanding are important. CGT comparability studies present some difficulties given the current level of maturity of the field, and some of the flexibility needed for such products is beyond what is currently addressed in ICH Q5E. And finally, discussions are needed among developers and regulators to overcome the current limitations for each CGT product modality.

Morning Session

Comparability Considerations for Viral-Vector–Based Gene Therapies: Demonstrating comparability for viral-vector–based gene therapies has become more straightforward than that for cell-based products, in part because standard manufacturing technologies (e.g., chromatography and filtration) are used and improved analytical testing methodologies have been developed. Advancements in the elucidation of structure–function relationships for AAV-based gene therapies have been critical in enabling additional characterization studies to support manufacturing changes.

Presented case studies made it clear that effectively demonstrating comparability relies on an in-depth understanding of a given product, on well-designed structure–function studies, and on reliable approaches to measure product quality and potency. One case study described substantial manufacturing changes made early in development to increase yield and scalability. Those changes included switching from a human-based adherent-cell culture system to an insect-cell–based suspension-cell culture system for vector manufacturing and certain downstream process changes for product purification. They were all major changes, which when implemented at the same time would make demonstration of comparability difficult. To implement those major changes successfully during early clinical development — and to mitigate the related risks to quality attributes — a comparability strategy was planned and executed. It focused on a matrixed approach that included the following elements:

- analytical comparisons that included release, characterization, and stability testing
- animal studies including nonhuman primate (NHP) toxicology studies to address potential impact to product safety and a head-to-head mouse study to address product efficacy/dose response before and after changes were made.

Limited analytical method-development concerns were addressed in that case, with side-by-side testing of pre- and postchange product and orthogonal testing for confirmation. Additional problems included limited availability of test materials, which was addressed by supplementing with data from process development (PD) lots generated under conditions that were not compliant with good manufacturing practices (GMPs). Such an approach aligns with recommendations in the draft comparability guidance issued by the FDA in summer 2023 (**2**).

A second case study reported on changing from a serum-dependent adherent cell culture to a suspension-adapted, serum-independent cell culture system for vector manufacturing in support of late-phase development and future commercial needs. That required rederivation of cell banks and simultaneous changes to downstream process steps, manufacturing scale, and manufacturing site. Such major manufacturing changes made during late stages of clinical development were derisked through an analytical comparability strategy that was grounded by historical process and product knowledge, with a testing plan that applied an advanced analytical toolbox. This case study also included strategies to adopt analytical release method changes accompanying the planned manufacturing changes. The presenter showed that adequate analytical method bridging studies can support the introduction of new assays late in product development for future quality control (QC) testing of late-stage and commercial lots.

CGTP Summit 2023

Organizing Committee

Diane Blumenthal (Dianthus Biopharma Consulting, LLC), Barbara Bonamassa (Italian Medicines Agency, AIFA), Kathy Francissen and Deep Shah (Genentech, a Member of the Roche Group), Leslie Nash (Health Canada), K.R. Poudel (Tune Therapeutics), and Zenobia Taraporewala (BioMarin Pharmaceutical)

Speakers

Session 1: Comparability Considerations for Viral Vector-Based Gene Therapies
Garrett Daniels (Prevail Therapeutics)
Taro Fujimori (Ultragenyx)
Julia O'Neill (Direxa Consulting)
Phillip Ramsey (Sangamo Therapeutics)

Session 2: Comparability Considerations for Cell-Based Therapies
Kedar Dave (Bristol Myers Squibb)
Mark DiMartino (Allogene Therapeutics)
Elizabeth Lessey-Morillon (FDA Center for Biologics Evaluation and Research)

Note that health authorities encourage developers to use precise, accurate, and sensitive assays that leverage current technological advances — e.g., moving from quantitative polymerase chain reaction (qPCR) to Bio-Rad's droplet-digital PCR (ddPCR) technology for product testing and demonstration of comparability. Applied assays should be scientifically sound and appropriate both for their intended purpose and the stage of clinical development. For late-phase studies, a qualified/validated activity-based potency assay should be in place for a comparability study.

Central to the demonstration of comparability is defining a meaningful difference between data from pre- and postchange products. Selecting appropriate statistical approaches to demonstrate a meaningful difference is often a vexing issue among developers of CGT products. In one presentation, that topic was approached holistically by highlighting that the assessment of comparability is more than "just numbers." The choice of statistical approach depends on the question that is asked. In practice, that would mean knowing what will be compared and how potential changes to product attributes will be measured. Whether to use a robust statistical methodology or descriptive summary statistics (describing sample size, mean/median, data spread/distribution, comparisons of graphed results, and so on) should depend in part on the size of data sets available. Generated manufacturing experience, which generally depends on the phase of clinical development, is likely to affect the choice of statistical approach for analyzing comparability data. The presenter emphasized the importance of considering all available data (including PD data) in the comparability analysis and health authorities with access to such data.

Together, the case studies and statistical considerations presented in the morning session underscored the value of comprehensive, multifaceted comparability studies for viral-vector–based gene-therapy products. The extent of a study and its design are influenced by

- the type of (major or minor) manufacturing changes planned
- associated risk assessments
- the (early or late) phase of development
- product and process understanding
- available analytical tools (and their suitability for detecting potential changes in product attributes)
- the appropriate statistical approach
- the extent of data available to demonstrate comparability (when studies will be conducted).

A stepwise approach should be adopted. One of the first steps in assessing the potential effects of a manufacturing process change is identification of product attributes that are likely to be affected by the planned manufacturing change(s). The resulting list of attributes then should guide design of a comparability study. For CGT products, a robust potency assay that demonstrates the mechanism of action (MoA) could be the most powerful tool to establish a correlation between patient outcomes (safety and efficacy) and PQAs. However, even with a reliable potency assay in place, demonstrating analytical comparability alone may be inadequate to ensure that pre- and postchange products will show similar safety and efficacy profiles in vivo. In some cases, regulators might request that additional in vivo studies be conducted — for example, if there is a reliable animal model to assess dose-response curves with pre- and postchange products. The discussion panel noted that nonclinical studies generally are less precise than analytical methods and in some cases actually could provide less valuable information than an in vitro assay would.

Afternoon Session

Comparability Considerations for Cell-Based Therapies: When it comes to applying the principles of comparability assessments as described in ICH Q5E, cell-based therapies present unique challenges for several reasons, including multifaceted manufacturing processes and the complexity of cells and the MoAs of cell-based products. Current understanding of the PQAs for cell-based therapies is limited, so it is difficult to identify which attributes would be relevant to product safety and efficacy. From a regulatory perspective, many cell-based therapies are highly variable by nature and thus can pose challenges for assessing manufacturing consistency and evaluating comparability to ensure that every patient receives a product of sufficient quality. Regulators recommend that product and process characterization and assay development begin early in a program and continue throughout a product's life cycle. Comparability study design depends on the risk posed by a given change, the stage of development at which it is made, and the potential effects on PQAs and interpretation of clinical study data.

Patient-derived starting materials are particularly heterogeneous for autologous cell-based therapies. Variability in cellular starting material affects both process performance and lot-to-lot product quality, so comparability studies need to account for that. For example, a known concern with autologous, lentiviral vector (LVV)–transduced CAR T-cell products is variability in their expression of CAR protein — not necessarily because of differences in the LVV, but rather in the incoming T cells themselves. Differences in the quality of starting material from patients are the primary reason why the length of a commercial CAR T-cell manufacturing process can be highly variable among patients (taking from one to three weeks). Such high variability in process duration affects CoGS, forecasts per run, and availability of manufacturing suites — and it ultimately can delay the availability of drug products for patients in advanced stages of disease.

A well-designed comparability package should use correlative analyses to parse out the contribution of starting-material variability from minor differences in product quality. In addition to building product understanding, such exercises in correlative analysis prepare a sponsor to present regulators with a data-driven case to address minor changes in product quality, which is not unusual with autologous cell-based therapies. Similar challenges are observed for allogeneic, donor-derived cell-based therapies, with which variability among donors (or even between donations from a given donor) affects the profile of

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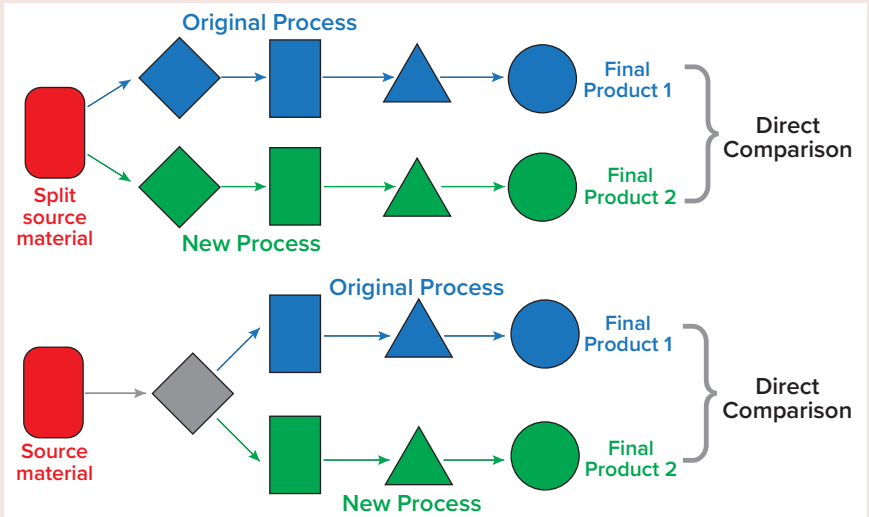
Figure 1: Paired study runs from the same donor material can reduce the complexity in designing comparability studies. (TOP) A typical paired study requires split postsource material because the intended change is introduced very early in the manufacturing process. (BOTTOM) In another paired study design, a paired split is introduced to streamline comparability after an intermediate step(s) (gray diamond). Keeping the donor constant in either case removes patient/donor variability from a comparability perspective.



cellular starting material and thus final product outcomes.

An afternoon case study provided a solution to account for patient/donor starting material variability. "Blocked" or paired-run studies (also known as *split manufacturing runs*) divide a manufacturing process at the cellular starting material (or further downstream in the process, depending on the nature of a change). Such studies using the same cellular starting material can help a sponsor establish comparability by removing patient/donor variability from the assessment (Figure 1). For autologous cell-based therapies, it might be necessary to use healthy donor cells as a surrogate starting material when patient-derived material is not available for such studies. This case study also emphasized the need for early planning of comparability studies and minimizing the number of studies necessary by addressing more than one process change at a time. When manufacturing changes are implemented during early clinical study phases, comparability studies can focus on demonstrating no adverse effect on safety.

In some cases, when significant changes are introduced after initiation of pivotal clinical studies, sponsors might have to expand the clinical trials to gather enough data from patients who received postchange products. That might be necessary for a true assessment of the impact on both the safety and efficacy of those products. Such expansions can increase costs, delay timelines, and make commercializing cell-based therapies fiscally unsustainable.

Learnings from the development of autologous cell-based therapies can be leveraged in development of allogeneic therapies, particularly regarding what constitutes a "better" starting material for manufacturing multiple batches from a small pool of donors. In manufacturing of T-cell products, early memory phenotypes, less differentiation, and overall cell health are generally considered to be critical for success. For ensuring consistent and successful manufacturing, such learnings also can be leveraged in selecting donors for allogeneic therapies.

At the summit, robust debate ensued about the proper use of statistical modeling in comparability assessments for cell-based therapy products. During the panel discussion, some limitations were pointed out about implementing statistical models for comparability assessments of cell-based products. In particular, their characterization is still evolving, so justification is currently inadequate for including potential critical quality attributes (CQAs) in statistical modeling. In addition, data sets might be too limited to be statistically meaningful.

Because CGTs are being developed for a global market, alignment is needed across regulatory agencies with regard to proper use of statistics and statistical modeling for demonstrating comparability of CGT products when manufacturing changes are made. **Successful implementation of an analytical comparability study requires comprehensive understanding of quality attributes and a product's MoA.** It is important to recognize the CGT industry's current level of maturity when discussing a "comprehensive" understanding of a product. The relatively limited understanding of CGTs compared with traditional recombinant-protein products is the primary barrier to developing an analytical approach for assessing comparability. Meaningful measures of potency in analytical comparability studies are crucial to verifying that in vivo activity is not compromised.

For now, a "fit for purpose" approach is needed for CGTs, given the limited product and process understanding combined with the necessity of manufacturing process changes. Building up comprehensive product understanding for cell-based therapies will require dedicated organizational efforts to integrate process and product knowledge. Many companies have a dedicated function to consolidate data from QC and characterization methods together with research and nonclinical studies as well as clinical samples to elucidate functional effects based on correlational analysis. Building a deep understanding of advanced therapies is likely to involve an extended learning process.

Leveraging deep CGT product understanding will be key to the future of comparability strategies for

- assessing the true impact on product quality before and after manufacturing changes
- defining the needed analytics to capture accurately the effects of proposed manufacturing changes
- ascertaining and ensuring that product coming from a new manufacturing process is highly similar to what has been evaluated in patients already — and that the existing knowledge is sufficiently predictive to ensure that any differences in PQAs

Disclaimer

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have no adverse effect on product safety or efficacy.

CGTs are some of the most complex drugs developed and manufactured to date. As discussed, complexities in the MoA and manufacturing of these products makes the impact of process changes on safety and efficacy difficult to quantify precisely. However, manufacturing changes must be made during development. Thus, the commercial success of CGTs will rely heavily on well-designed comparability exercises that can address the effects of manufacturing changes adequately using information available now. Advanced-therapy (CGT) sponsors should continue to build the analytical tools necessary to gain a deeper understanding of these products to enable future development.

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continued from ***page 11***

Standardization and Optimization

Several industry trends are changing the needs of advanced-therapy developers, increasing the urgency of developing collection standards to optimize product quality. Autologous therapy manufacturing is shifting toward decentralized operations that could change the way leukopaks are managed postcollection. And as more allogeneic approaches enter clinical testing, subtype varieties are increasing, making cell-selection processes even more important. Such subtypes include γδ T cells, which lack immune markers and must be filtered cleanly from both the more populous αβ T cells and natural killer (NK) cells, which all share a similar phenotype.

Therapeutic developers may decide to refine their screening criteria as clinical data come in. Blood centers often are essential partners in this process. They can support additional characterization and testing of donors and assess implementation of new criteria. Although well-versed in protocols, some blood centers also can identify nonoptimal processes and advise companies on how to refine their collection protocols. The right partners start with the right questions: Would increasing the donor age cutoff make it easier to find a suitable donor? Do existing data support restrictions such as cytomegalovirus-negative–status criteria? If time requirements are strict, should we expand the potential population by adjusting the body mass index (BMI) cutoff?

Savvy developers express a growing interest in adaptive protocols, and blood centers are well positioned to advise on characteristics based on their experience in apheresis. Such protocols might include establishing decision trees for troubleshooting and built-in contingencies. For example, developers could introduce alternative processes when clumping occurs in an apheresis product or additional methods to meet cell-count targets within optimal apheresis times.

Large-scale genomic and population health studies, especially the National Institutes of Health (NIH) All of Us Research Program, can provide broad insights and guide standardization efforts. Such programs better elucidate factors that influence health, and such data someday could be used to correlate inputs from a patient or donor with therapeutic outcomes.

That correlation will be key for the CGT field to mature fully. Apheresis provides bedrock starting material for advanced-therapy manufacturing. By increasing developers' understanding of the factors that have the biggest impact on safety and efficacy, process optimization at the earliest stages of development will enable better outcomes for tomorrow's patients.

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Lessons in Bioreactor Scale-Up

Part 1: Exploring Introductory Principles

Muhammad Arshad Chaudhry

Biologics such as monoclonal antibodies (mAbs), other recombinant proteins, and viral vectors now represent a major class of pharmaceuticals. Their manufacturing, based primarily on mammalian-cell culture, has advanced significantly in the past two decades. Heat and mass transfer, fluid dynamics, reaction kinetics, and other chemical-engineering principles apply broadly to biologics development and production. For cell-culture–based processes, development scientists and engineers use such principles to optimize transport of nutrients (including oxygen) to cells, mixing and removal of undesired metabolites, and collection and purification of molecules of interest.

Cell-culture process development involves investigation of both scale-dependent and scale-independent bioreactor parameters. Scale-independent parameters — e.g., pH, temperature, dissolved oxygen (DO) concentration, and media composition and osmolality — typically are tested and optimized in small-scale bioreactors, then kept constant during scale-up. Scale-dependent parameters, on the other hand, are affected by a bioreactor's geometric configuration and operating parameters. Bioreactor-impeller rotational speed (N), gas-sparging rates, working volume, and so on affect the state of fluid flow and mixing (homogenization) in a bioreactor, thus influencing the physical forces that act on cells. Hence, bioreactor scale-up requires optimization of operating parameters for the large-scale bioreactor to be used in a production process.

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Bioreactor scale-up influences physical, chemical, and biological factors of cell culture. Physical factors, as mentioned above, include bioreactor configuration, aeration, agitation, heat transfer/removal, and mixing. Chemical factors affected by scale-up include type and concentration of acid/base needed for pH control, water quality, and foam formation caused by surface tension changes. Biological factors include the number of generations associated with inoculum development and production phases, mutation probability, contamination vulnerability, and elimination of selection pressure (**1**).

A biologic manufacturing process typically involves scaling up of production from miniaturized, high-throughput bioreactors (15–250 mL) to bench-scale glass or single-use reactors (1–10 L) and then pilot- and production-scale bioreactors (200–5,000 L or larger). Bioreactor scaling is not trivial; it is a difficult and complex task that requires a delicate balance between equipment design and operational capabilities, which often vary considerably across scales, to provide similar hydrodynamic and mass-transport conditions for cell growth and production. Several factors contribute to the difficulty of scaling. They include the complexity of biological systems and the heterogeneous (hydrodynamic and mass-transfer) environments prevailing in large-scale bioreactors leading to substrate and pH gradients. Such complexity leads to variations in cell growth, metabolism, protein production, and sometimes, product-quality profiles across scales — commonly known as *process variability* due to bioreactor scaling.

Because single-use bioreactors are now commonplace, many contract development and manufacturing organizations (CDMOs) and other commercial manufacturers often have production bioreactors located at many manufacturing sites across a country or even around the globe. Those bioreactors can differ not only in their geometric configurations — e.g., in their bioreactor height:tank diameter (H/T) and impeller diameter:tank diameter (D/T) ratios — but also in their sparger configurations and impeller types. Many suppliers of single-use bioreactors,

Product Focus: Recombinant proteins, viral vectors

Process Focus: Cell culture

Audience: Manufacturing and process development

Keywords: Bioreactor geometries, fluid dynamics, mixing and gassing strategies

Level: Intermediate

Figure 1: Bioreactor geometric similarity and change in volume at a constant scale-up factor; D = impeller diameter, H = bioreactor height, T = tank diameter, 1 = small-scale vessel, 2 = large-scale vessel

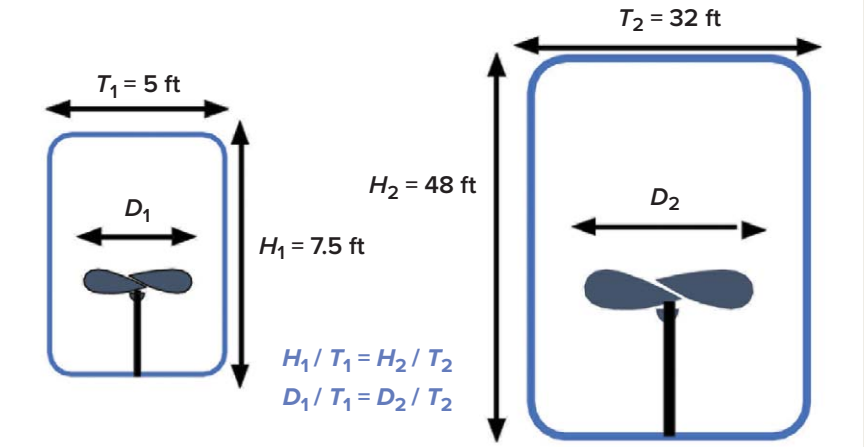
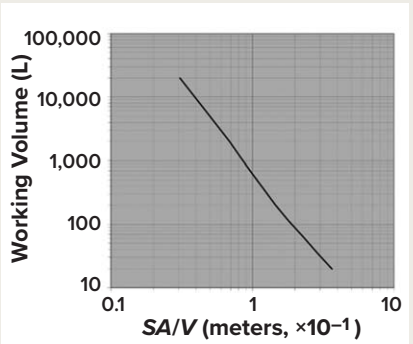


Figure 2: Changes in surface area per unit volume (SA/V) with increase in bioreactor size; adapted from Marks (**2**)



however, now provide a "family" of bioreactors with geometrically similar designs at working volumes that span from development (10–50 L) to pilot and production scales (200–2,000 L).

Key Considerations in Bioreactor Scale-Up

Key factors encountered during bioreactor scale-up include

- nonlinearity
- differences in (bio)chemical equilibrium
- differences in fluid dynamics and agitation settings
- development of temperature, oxygen, pH, and CO_2 gradients
- material properties and equipment selection.

Nonlinearity in Bioreactor Scaling: *Geometric similarity* — maintaining similar H/T and D/T ratios across bioreactor sizes — is often a prerequisite for scale-up (Figure 1). To accomplish that, all length dimensions are scaled proportionally. Laboratory-scale bioreactors often have an H/T ratio of 2/1. Large-scale bioreactors, on the other hand, can range in ratio from 2/1 to 4/1. Meanwhile, D/T ratios generally are maintained in the range of 1/3 to 1/2.

One consequence of holding H/T ratios constant during scale-up is a dramatic reduction in the ratio of surface area to volume (SA/V) (Figure 2) (**2**). Doing so also increases bioreactor volume dramatically. As Figure 1 illustrates, maintaining an H/T value of 1.5 and using a scale-up factor of 6.4 changes the volume from 147 ft^3 in the small bioreactor to 38,604 ft^3 at the larger scale — a 26-fold increase.

The resulting decrease in SA/V ratio creates challenges for heat removal in large-scale microbial fermenters. For animal-cell–culture bioreactors, CO_2 removal becomes an issue at large scales because of the increase in bioreactor height, decrease in liquid surface area at the top, and added head pressure from the higher liquid height. All those factors decrease the efficiency of CO_2 stripping in the bioreactor overlay. Scale-up generally results in higher shear-force variation with less efficient bulk mixing because of longer circulation times and larger stagnant areas.

Many other parameters change during scale-up, such as a transition from laminar to turbulent flow regimes. Table 1 shows interdependence among several scale-up criteria based on a scale-up factor of 125. It is evident from the table that the bigger the scale-up factor is, the more that the process parameters — and scale-up criteria — change. An analysis of those data makes it clear that even if geometric similarity (H/T) is maintained during scale-up, then conditions in a large-scale bioreactor never will exactly duplicate those in a small-scale bioreactor because of changes in parameter values.

Adequate mixing in large-scale bioreactors generally requires an extremely high power input, which is infeasible either because of equipment-size and mechanical constraints or shear sensitivity in mammalian cells. Consequently, substrate and pH gradients generally occur in large-scale bioreactors. Both product yields and product-quality profiles can be different in large-scale bioreactors.

In addition to maintaining H/T ratios, process scientists must determine other design parameters to be within acceptable ranges using the ratios of the impeller diameter (D), impeller-bottom clearance, liquid height, vessel height, and baffle width to the vessel diameter. Traditionally, one of the following scale-up criteria or combinations thereof have been used in microbial and animal-cell culture scale-up:

- a constant impeller-tip speed
- a constant power per unit volume (P/V)
- a constant oxygen mass-transfer coefficient (k_La)
- a constant mixing time
- a constant Reynold's number (Re)
- a constant gas-flow rate per unit volume (vvm)
- a combination of these criteria, such as constant P/V and vvm or a constant k_La and gas-flow rate (or gas velocity) across scales (**3**).

Table 1 lists parameters considered during scale-up, all of which depend on tank diameter and impeller rotational speed (D and N values, respectively). Fixing those values also fixes all other quantities used in scale-up calculations. Because power input, impeller-tip speed, Reynold's number, and the like have different dependencies on D and N values, a change in those numbers (due to a change in scale) also will alter the physical environment in which cells are cultured. Such changes can affect the distribution of chemical species and, in extreme cases, injure or kill cells. In

Table 1: Interdependence of scale-up parameters; the "scale-up criterion" column indicates which variable is held constant between two scales. Table contents are adapted from Lara et al. (**4**).

			Production Bioreactor (10^4 L)			
Scale-Up Criterion	**Designation**	**Small-Scale Bioreactor (80 L)**	**Constant *P*/*V***	**Constant Impeller Rotation (*N*)**	**Constant Tip Speed**	**Constant *Re***
Energy input	$P \propto N^3 D^5$	1	125	3,125	25	0.2
Volumetric energy input	$P/V \propto N^3 D^2$	1	1	25	0.2	0.0016
Impeller rotation	N	1	0.34	1	0.2	0.04
Impeller diameter	D	1	5	5	5	5
Impeller-pump rate	$Q \propto N D^3$	1	42.5	125	25	5
Circulation time	$t_c \propto V/Q$	1	2.94	1	5	25
Impeller-tip speed	$V \propto N D$	1	1.7	5	1	0.2
Reynold's number	$Re \propto N D^2$	1	8.5	25	5	1

α: alpha factor (process specific) **D**: impeller diameter **N**: impeller rotation speed **P**: impeller power **Q**: pump rate **Re**: Reynold's number
t_C: circulation time, calculated as the inverse of the volumetric pump rate of the impeller; $t_c \sim (t_m \div 4)$, in which t_m is mixing time **V**: volume

addition, cellular metabolic responses can change from one scale to another, which ultimately would result in a change in cell physiology.

It is clear from Table 1 that scaling up when maintaining equal *P*/*V* ratios results in lower impeller speeds but higher tip speeds, longer circulation times, and greater $k_L a$ values. On the other hand, scale-up based on impeller speed (*N*) is not feasible because doing so would decrease *P*/*V* ratios substantially. Scale-up based on equal *Re* values is also infeasible because *P*/*V* would be reduced by a factor of 625.

Circulation time — the average time that a particle requires to travel from and return to a fixed location in the bioreactor, a parameter that is directly proportional to mixing time — generally is not used as a primary scale-up criterion. Except when using equal impeller rotation speeds (*N*) across bioreactors, all other scale-up criteria increase circulation time. Holding circulation time constant results in a 25-fold increase in *P*/*V*. Similarly, scale-up based on equal tip speed reduces *P*/*V* ratios by a factor of 5 and also would result in lower $k_L a$ values. In addition, circulation time increases fivefold.

In summary, the scale-up issues discussed above all relate to transport phenomena: heat, mass, and momentum transfer. Scale-up generally involves moving processes being controlled by cell kinetics at laboratory scale to control by transport limitations at larger scales. Consequently, time scales for mixing and reaction rates become important in determining the level of homogeneity in a large-scale bioreactor. Therefore, the objective of a scale-up exercise is not to keep scale-dependent parameters constant; rather, it is to define the operating range(s) of scale-sensitive parameters such that cellular physiological states — and thus cell-line productivity and product-quality profiles — can be maintained across scales.

The objective of a scale-up exercise is not to keep scale-dependent parameters constant; rather, it is to define the operating range(s) of scale-sensitive parameters such that cellular physiological states — and thus cell-line productivity and product-quality profiles — **CAN BE MAINTAINED** across scales.

Biochemistry and Chemical Equilibrium: Most chemical reactions, including cellular metabolism, require good mixing to have uniform reactant and/or substrate concentrations. Such processes also can release heat as components react to form products. Cellular — and especially microbial — metabolism generates heat that must be removed from a bioreactor to maintain constant temperature. Changes in *SA*/*V* ratio upon scale-up can influence heat-transfer efficiency and, in turn, affect how reactions proceed inside a large bioreactor. Cells generally prefer narrow ranges in temperature, pH, osmolality, and substrate concentrations for optimal performance. Hence, large-scale processes must be optimized to provide the best possible environment for cell growth and biologic production.

Mixing, Agitation, and Fluid Dynamics: As discussed above and as shown in Table 1, scale-up based on equal *P*/*V* values increases circulation time (and hence mixing time) by almost threefold. Consequently, environmental heterogeneities such as substrate, pH, and oxygen gradients can occur in large-scale bioreactors. As cells travel through such gradients, they will be exposed to a continually changing environment, which can alter overall culture performance. Mixing times are higher in animal-cell bioreactors than in microbial fermenters because, in the former case, low to moderate power input must be applied. In large-scale cell-culture bioreactors, mixing times can be in the order of minutes (**4**).

Fluid dynamics change nonlinearly as bioreactor size increases. Hence, transitions from laminar- to turbulent-flow conditions occur, and generally such changes are difficult to predict. Keeping flow at the correct *Re* value (which helps to predict laminar- and turbulent-flow conditions) enables a culture process to maintain thermal and mixing efficiency. The type of mixing regime needed for a given process depends on whether cells are adherent or flow freely in suspension. During

scale-up, *Re* values often change based on other scale-up criteria; hence, a mixing regime can change from laboratory to clinical and commercial scales, complicating successful scale-up.

Development of Gradients in Large-Scale Bioreactors: Low power input to large-scale cell-culture bioreactors generally results in longer mixing times, which can cause stagnant zones to develop. While circulating in a bioreactor, cells therefore can experience transient changes in environmental parameters such as pH levels and oxygen concentrations. Such transient excursions in a bioreactor environment can influence cellular physiology. By-products of mammalian-cell metabolism (e.g., lactate) accumulate over the culture period and, if mixing is inadequate, will generate zones of low pH in the bioreactor. Similarly, a transient increase in metabolic activity can cause oxygen depletion in high-nutrient concentration zones.

To summarize, bioreactor parameters interact in complex ways because of heterogeneities in large-scale vessels, and a gradient in one culture variable can originate gradients in other variables (**4**). Such effects can alter cell phenotypes or diminish genetic stability of the plasmid or vector containing a recombinant gene of interest.

Oxygen is sparingly soluble in water and hence must be supplied continuously during cell culture. Mixing times (in the order of minutes) can spur on development of oxygen gradients in large-scale bioreactors. Lara et al. postulate that, in a mammalian-cell stirred-tank bioreactor of 20 L, oxygen gradients can occur if cell concentrations exceed 20 million cells/mL of culture fluid (**4**). Such concentrations are relatively common in large-scale bioreactors operated in fed-batch mode.

Because of the dynamic nature of mammalian-cell metabolism in stirred-tank reactors, operated either in a batch or fed-batch mode, **culture pH** decreases over time due to accumulation of acidic by-products. Bioreactor pH, therefore, needs to be controlled by the addition of a base. But poor mixing could give rise to a high-pH zone near the base addition port. The pH level in such zones can rise transiently to nonphysiological values, affecting not only cellular metabolism, but also intracellular pH, changes in which can affect enzyme activity. For example, a change in intracellular pH can alter glucose transport, ratios of adenosine triphosphate (ATP) to adenosine diphosphate (ADP), and product-quality profiles.

During a mammalian-cell fed-batch culture, the increase in biomass concentration over time results in production of **CO_2** as a by-product of metabolism. Compared with oxygen, CO_2 has higher solubility in culture media, and depending on culture pH, it can be absorbed as dissolved CO_2 (dCO_2). In an ideal situation, depending on air-sparging rates and bubble sizes, most dCO_2 is stripped by air and is removed from the bioreactor as part of the exit-gas stream. However, the decrease in SA/V ratio during scale-up diminishes gas exchange on the liquid surface and hampers dCO_2 stripping by air flowing through the overlay or headspace. Thus, in large-scale bioreactors, dCO_2 is higher than it is in laboratory-scale vessels because the increased liquid column height also increases head (hydrostatic) pressure. Heightened hydrostatic pressure at the bottom of a bioreactor increases gas solubility and results in accumulation of dissolved gases (**1**).

Accumulation of dCO_2 has several effects on culture conditions, including changes in culture pH due to CO_2 being in equilibrium with sodium bicarbonate in the medium. That generates carbonate and bicarbonate ions, both of which decrease culture pH, thereby changing intracellular pH (**5–8**). Such ions also increase osmolality at constant bioreactor pH (**9**). Gray et al. report that air bubbles of 2–3 mm and a sparging rate of 0.005 vvm are required to operate a 500-L Chinese hamster ovary (CHO)–cell perfusion bioreactor without oxygen limitation and dCO_2 accumulation (**10**).

Material Properties and Equipment Selection: Physical and/or chemical properties of the material of construction used in small- and large-scale bioreactors can influence not only a culture's progress, but also its outcomes. Single-use and high-throughput bioreactors have become commonplace for laboratory-scale experimentation. Now, many commercial-scale cell-culture processes are carried out in single-use bioreactors of different sizes. Commercial-scale processes developed in the 1990s were performed in stainless-steel bioreactors of multiple-thousand liters in scale. For such equipment, cleaning validation is a requirement. Because of improvements in cell-culture media and bioprocess optimization over the past two decades, most modern commercial processes are performed at 2,000-L scale.

How To Scale Up — The Role of Process Parameters

Process engineers and development scientists can perform bioreactor scale-up using either agitation- or gassing-based parameters.

Mixing time, P/V ratio, and impeller-tip speed are parameters that fall under the category of **agitation-based scaling parameters**. Because those parameters depend on agitation, they cannot all be held constant during scaling. Keeping mixing time constant generally necessitates very high P/V values, whereas keeping tip speed constant might require low agitation rates that could create oxygen-delivery issues. Hence those parameters need to be evaluated individually, and final scale-up agitation must result in acceptable values for all three.

Power input per unit volume (P/V) refers to the amount of power transferred by the agitator motor through the agitator shaft and impellers to a given volume of cell-culture broth. P/V is calculated using Equation 1:

$$P/V = P_0 \times \rho \times N^3 \times D^2$$

Therein, P_0 represents the impeller power number, N is the impeller speed, D is impeller diameter, and ρ denotes the liquid density. To scale up based on maintaining P/V constant across scales implies Equation 2,

$$(P_0 \times \rho \times N^3 \times D^2)_2 = (P_0 \times \rho \times N^3 \times D^2)_1$$

in which subscripts $_2$ and $_1$ refer to large- and small-scale bioreactors, respectively.

Liquid density (ρ) can be held constant if a culture medium will not change during scale-up. The impeller power number (P_0) also can be

considered constant when preserving geometric similarity across scales, assuming fully developed turbulent flow, and using the same impeller type, configuration, and spacing.

Equation 2 can be simplified to Equation 3:

$$(N^3 \times D^2)_2 = (N^3 \times D^2)_1$$

And large-scale rotational speed (agitation) can be calculated using Equation 4:

$$N_2 = N_1 \times (D_1 \div D_2)^{2/3}$$

As that equation shows, at large scales, the agitation rate must be decreased proportionally to the impeller diameter ratio to 2/3 power.

Impeller-Tip Speed: Tip speed serves as a measurement of the shear rate produced by impeller blades in a cell-culture medium. Using tip speed as a primary scale-up criterion generally leads to a decrease in P/V and hence is used infrequently for animal cell culture. However, Equation 5 can be used to calculate the impeller rotation speed (agitation) at the large scale:

$$\pi \times D_2 \times N_2 = \pi \times D_1 \times N_1$$

That can be rearranged to Equation 6,

$$N_2 = N_1 \times (D_1 \div D_2)$$

The latter equation shows that, at the large scale, agitation must be decreased proportionally to the impeller diameter.

Mixing time represents how much time is needed for bioreactor content to attain a homogeneous state. The parameter depends on both the level of turbulence within and bulk turnover of that content. Mixing times on the order of 160 seconds have been reported for large-scale (100 m^3) fermenters (**1**). Inefficient mixing in a large cell-culture bioreactor can generate pH, oxygen, and substrate gradients. Mixing time also depends on impeller speed (agitation rate), impeller type and size, impeller spacing from the bottom of the vessel, and baffle design (if applicable). When using equal mixing time as the primary scale-up criterion, Equation 7 can be used to determine the impeller rotation speed for the large-scale bioreactor (with $_2$ and $_1$ again representing large and small vessels):

$$N_2 = N_1 \times (D_1 \div D_2)^{1/4}$$

Again, using equal mixing time, agitation in a large-scale reactor must be decreased proportionally to the impeller diameter to the power of 1/4. Because scale-up based on equal mixing time results in large P/V values, it is used infrequently as a scale-up criterion for mammalian-cell bioreactors.

The above analysis shows that it is not possible to keep all the discussed scaling parameters constant. Scale-up still is performed based on industry experience, knowledge gained during process development, and when available, familiarity with the historical performance of a target bioreactor.

Gassing-Based Parameters: During bioreactor scale-up, liquid height:tank diameter ratios often can be >1 for the larger vessel, limiting back-mixing of upward-moving sparged gas. Consequently, vertical DO gradients will occur if bulk mixing is slower than the oxygen mass-transfer rate, and radial DO gradients will emerge because shear rates decline rapidly with increased distance from the impeller (**1**). Hence, in large-scale bioreactors, the most important — and most difficult — aspect of a scale-up exercise often is providing sufficient oxygen to the culture while maintaining an oxygen-transfer rate (OTR) equivalent to that of the small-scale vessel. Successful scale-up based on providing a constant OTR depends on the ability to measure or estimate k_La values and the gassed power per unit liquid volume (P_g/V).

It is not possible to keep all the discussed scaling parameters constant. Scale-up still is performed based on industry **EXPERIENCE, KNOWLEDGE** gained during process development, and when available, **FAMILIARITY** with the historical performance of a target bioreactor.

Scale-up based on a constant OTR assumes that the oxygen uptake rate (OUR) in a bioreactor equals the OTR, as shown in Equations 8 and 9:

$$\text{OTR} = k_La \times (C_{sat} - C_L)$$

$$\text{OUR} = \mu \times X \times Y_{X/O2}$$

Therein, C_{sat} denotes the bioreactor DO concentration at saturation, C_L represents the measured DO concentration (at any time), μ is the specific growth rate, X is the measured cell density, and $Y_{X/O2}$ is the *yield coefficient* (the calculated cell yield per amount of oxygen consumed).

Changes in back-pressure and hydrostatic pressure will influence C_{sat} and subsequently C_L values. In tall bioreactors, local and saturation concentrations of oxygen can differ at the top and bottom of the vessel. To account for such differences, the log mean of the DO concentration difference should be used. Because the critical DO concentration that adversely affects growth rate is low in most aerobic cell cultures, C_L is assumed to be zero.

Available literature provides several empirical correlations of the general form (Equation 10)

$$k_La = C(P_g/V)^{\alpha} \times V_s^{\beta}$$

to estimate k_La using gassed power per unit volume (P_g/V) and gas superficial velocity (V_s). Values for the constant (C) and exponents (α and β) are process-specific, and a broad range of values have been reported. Generally, the dependence of k_La on P_g/V decreases with increase in scale.

Such correlations have been reported for multiple gas–liquid systems and bioreactors (**11**). However, exercise caution when using those correlations for bioreactor scale-up because doing so can generate significant errors.

Although both k_La and C_L can vary substantially during a fed-batch run, the minimum acceptable value of C_L is generally known (based on experiments in laboratory-scale bioreactors). A C_L value >30–70% saturation generally ensures adequate DO concentrations in

less-mixed regions of a bioreactor (**1**). That said, scale-up based on maintaining constant DO across scales can be tricky, and engineers and development scientists must consider methods to control DO such as agitation, air and oxygen flow rates, air enrichment with oxygen, and even use of pure oxygen. Cascade control using such methods is generally effective in maintaining DO above critical values.

Maintaining $k_L a$ with either equal vvm (volumetric gas-flow rates) or equal V_s (superficial gas velocity) across scales is a common scale-up principle for mammalian-cell bioreactors. Both principles result in similar P/V values across scales and have been applied successfully in commercial production processes. When basing scale-up on vvm, V_s values also should be calculated and checked to ensure that resulting values are reasonable.

A process must balance vvm and V_s values. If vvm is kept constant, then V_s can increase to the point of flooding the impeller in a production-scale bioreactor (**1**). If it is advantageous to hold V_s constant during scale-up, the vvm value should be larger at the smaller scale than at the target scale. Although vvm has little effect on the value of $k_L a$, higher air-flow rates can increase foaming dramatically and increase gas hold-up. If both P_g/V and V_s are kept constant during scale-up, then gas hold-up does not change with scale-up.

Single-use bioreactors often are designed with unique (proprietary) impellers. Those can be bottom-mounted or attached using a shaft with an offset from the vessel center to improve mixing. In such instances, experimental data must be collected to identify P/V values and gas-flow rates that will meet target $k_L a$ values — and thus satisfy oxygen demands and ensure solution homogeneity.

In large-scale bioreactors, air sparging is used both for oxygenation and CO_2 stripping. Whereas oxygen transfer depends on superficial gas velocity, CO_2 removal is dependent on total gas-flow rates (vvm). Therefore, scaling based on vvm values can result in inadequate CO_2 stripping. That is generally a concern for bioreactors of ›2,000 L. Xu et al. discuss strategies for ensuring proper oxygenation and CO_2 removal (**12**).

Continued Exploration

This article represents the first in a series of explorations into bioreactor scale-up. Read the April 2024 issue of BPI to find the next installment, which will overview fundamental aspects of fluid flow and mixing in bioreactors.

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A Methodology for Identifying Current and Future Skills Gaps

Future-Proofing the Bioprocessing Sector As It Embraces Industry 4.0 Principles, Part 1

Jason Beckwith, Paul Rooney, Gregor Adams, Stephen Goldrick, William Nixon, and Stavros Kourtzidis

The biopharmaceutical industry is at the forefront of technological advancements, with "biopharma 4.0" revolutionizing the way biomanufacturing is conducted (**1**). The concept combines automation, artificial intelligence, data analytics, and real-time monitoring to enhance efficiency, flexibility, and quality in bioprocessing (**2**). However, such rapid evolution has given rise to a significant skills gap, with the existing workforce lacking competencies needed to leverage the full potential of this transformative paradigm (**3, 4**). In this two-part report, we explore the key expertise required for the transition and the resulting skills gaps, with discussion of strategies for addressing these talent challenges.

Biopharma 4.0 demands a new set of skills and expertise to harness the power of digitization and automation most effectively (**5, 6**). Key needs include experience with data analysis, machine learning (ML), process optimization, and cybersecurity — in addition to advanced knowledge of complex bioprocessing technologies. Biopharmaceutical professionals also must possess adaptability, critical-thinking skills, and problem-solving abilities to navigate the rapidly evolving landscape of biopharma 4.0 (**7, 8**). This new era brings tremendous opportunities for increased productivity, efficiency, and innovation in drug development and biomanufacturing (**9**). However, as the industry rapidly evolves, it faces unique challenges in acquiring and developing the talent needed to harness the potential of biopharma 4.0 (**10, 11**).

Biopharma 4.0 encompasses a breadth of technological innovations such as artificial intelligence (AI), ML, big-data analytics, robotics, and advanced manufacturing techniques (**12, 13**). To bridge the associated skills gap, the industry first must identify **core competencies** required for biopharma 4.0 (**14**). With *digital fluency,* professionals possess a strong foundation toward *digital literacy,* including proficiency in data analysis, cloud computing, and cybersecurity (**15, 16**). Familiarity with data integration, management, and interpretation is crucial for harnessing the power of digital technologies in bioprocessing (**17**).

Similarly, competency in **data analytics** includes the ability to analyze and derive meaningful insights from large data sets — a key skill in biopharma 4.0. Professionals should be adept at using advanced analytics tools, predictive modeling techniques, and data visualization to optimize processes, improve decision-making, and drive innovation (**5, 18**). **Automation and robotics** functions as another competency for biopharma 4.0, which relies on such technologies to enhance operational efficiency, accuracy, and scalability. Professionals should be equipped with the knowledge of programming, operating, and maintaining automated systems in addition to the ability to integrate them seamlessly into bioprocessing workflows (**17, 19**).

PRODUCT FOCUS: ALL BIOPHARMACEUTICALS

PROCESS FOCUS: MANUFACTURING

AUDIENCE: OPERATIONS, HUMAN RESOURCES, MANAGEMENT, INFORMATION TECHNOLOGY

KEYWORDS: BIOPHARMA 4.0, DATA SCIENCE, AGILITY, SENSORS, AUTOMATION, CYBER

LEVEL: INTERMEDIATE

Regulatory compliance is a competency in itself because adhering to guidelines and maintaining compliance are critical in the biopharmaceutical industry. Professionals should be well-versed in regulatory requirements related to data integrity, cybersecurity, quality control (QC), and good manufacturing practices (GMPs) in the context of biopharma 4.0 (**3**). Finally, life-science professionals must possess an **agile mindset** because biopharma 4.0 is characterized by rapid technological advancements. Professionals should be able to embrace change and continuous learning. The ability to adapt quickly to new tools, methodologies, and industry trends is essential for success in this dynamic landscape (**20, 17**).

Supply and Demand: As the industry rides the transformative wave of biopharma 4.0, it faces a critical challenge in the associated skills gap (**21**). The demand for talent equipped with the necessary skills to navigate the complexities of biopharma 4.0 far exceeds the current supply (**22**). This gap can be attributed to several factors. First, the **swift pace of technological advancements** has outpaced development of relevant educational programs and training initiatives, leaving a void in skills acquisition for rapid technological advancements (**23**). Second, the **disconnect between academia and industry** hampers timely development of curricula and training programs that align with the evolving demands of biopharma 4.0, described herein as *limited integration of academia and industry* (**24, 25**). Finally, **resistance to change** slows the adoption of new technologies and processes within many organizations, impeding the acquisition of necessary skills and hindering the implementation of biopharma 4.0 initiatives (**10**).

Collaboration between industry and academia will be crucial to bridging the skills gap. Industry experts can contribute to curriculum development, ensuring that educational programs incorporate biopharma 4.0 concepts, practical training, and internships. Such collaboration can facilitate the transfer of knowledge and help to ensure that graduates will be equipped with the relevant skills (**26, 27**).

Table 1: Expertise in biopharma 4.0 technology segment and keywords for Lightcast database searches; SQL = structured query language, RCA = root-cause analysis, DevOps = software development and information technology (IT) operations

Skills	Keywords	Skills	Keywords
Data Science	Data science + big data	Cyber	Cyber + cloud computing
	Data science + statistics		Cyber + agile
	Data science + Python		Cyber + DevOps
	Data science + SQL		Cyber + Internet of Things
	Data science + R		Cyber + security
			Cyber + solution architecture
			Cyber + virtual
			Cyber + web
Automation	Automation + robotics	Sensors	Sensors + batch manufacturing
	Automation + lean manufacturing		Sensors + continuous manufacturing
	Automation + process technology		Sensors + digital twin
	Automation + quality management		Sensors + electrochemical
	Automation + RCA		Sensors + embedded software
	Automation + simulation		Sensors + optical
	Automation + virtual reality		Sensors + process analytical technology
	Automation + continuous improvement		Sensors + spectroscopy

Herein we describe a multifaceted approach to bridging the biopharma 4.0 skills gap. First, employers should invest in comprehensive training and upskilling programs to equip the workforce with biopharma 4.0 competencies. Such programs can range from workshops and seminars to certifications and online courses. Encouraging employees to take part in continuous learning opportunities can enhance their skills and keep them up to date with the latest industry trends (**28**). Biopharmaceutical companies also should invest in comprehensive professional development programs to enhance workforce skills. Those programs could include targeted training programs, workshops, and certifications to upskill employees in areas such as data analytics, automation, and regulatory compliance (**28**).

Employers also would benefit from adopting innovative recruitment strategies to attract talent with biopharma 4.0 skills. That might involve partnering with specialized recruitment agencies. Additional strategies could include proactive talent sourcing, in which bold and enterprising organizations recognize and acquire talent with the adequate skill sets for industry 4.0. Companies can leverage online platforms, professional networks, and partnerships with educational institutions to connect with potential candidates (**29**). Skills assessments and gap analyses are crucial to helping each organization understand its specific requirements and enabling development for targeted training programs and recruitment strategies to address those gaps (**30**). To bridge the skills gap and prepare existing employees for industry 4.0, organizations should invest in upskilling and reskilling programs (**31, 28**). Such initiatives include training workshops, online courses, mentorship programs, and certifications to enhance employee knowledge and capabilities. Companies should highlight their commitment to technology advancements, innovation, career development opportunities, and a supportive work culture aligned with the principles of industry 4.0 (**32, 33**).

Finally, organizations should focus on nurturing internal talent through mentoring programs, cross-functional training, and job rotations. This approach enables employees to develop new skills, broaden their knowledge base, and contribute to diverse projects within a company. Offering financial support for further education or advanced degrees also can motivate employees to acquire specialized skills (**1, 34**).

In our research we have sought to define and identify the specific expertise and skills-gap requirements relevant to the biopharma 4.0 transition and to help the industry implement strategies for mitigating operational disruptions in the crucial manufacturing of drug products. To determine potential skills gaps related to bio/industry 4.0, we used the Lightcast labor analytics database to identify real-time labor-market trends and the occurrence rate for specific skill and expertise terminology, executing a customized search by applying different filters.

Table 2: Technology sector job titles

Sensor	Cyber	Data Science	Automation
Sensor Engineer	Cybersecurity Analyst	Data Scientist	Automation Engineer
Sensor Systems Engineer	Ethical Hacker/Penetration Tester	Data Analyst	Controls Engineer
Sensor Applications Engineer	Security Engineer	Machine Learning Engineer	Robotics Engineer
Sensor Technician	Security Operations Center Analyst	Data Engineer	Process Engineer
Sensor Sales Engineer	Security Consultant	Data Architect	System Integration Engineer
Sensor Quality Assurance Engineer	Information Security Manager	Business Intelligence Analyst	Industrial Engineer
Sensor Firmware Engineer	Network Security Engineer	Statistician	Maintenance Engineer

A *skills gap* is defined as the disparity between employers' requirements for experienced talent and the expertise offered by the available workforce. In this case, *demand* is defined as the manifestation of a specific skill in job postings, and *supply* is the frequency of a skill in the workforce profiles. Thus, the skills gap manifests the contrast between demand and supply. By measuring expertise in the available biopharmaceutical talent pool against the current demand, we tested the hypothesis of a potential mismatch of skills that has yet to be addressed by academia or training providers. We can predict the requisite expertise and compare that with skill sets represented in the available talent pool by measuring the pace of adoption for specific technologies in the biomanufacturing sector (**35**).

That study provides a quantitative analysis of biomanufacturing skills gaps at an expertise level, illustrating the key talent dynamics identifying their correlation with the rate of industry 4.0 adoption across all bioindustries. In addition, our research provides a novel measurement of the current biopharma 4.0 talent supply. The methodology is adapted from Li et al. (**3**), who analyzed the data-science and statistical-tools skills gaps in today's automotive manufacturing workforce using Emsi labor analytics. In 2015, Deloitte and The Manufacturing Institute forecasted an impending skills gap related to the introduction of smart manufacturing. Previous studies have attempted such measurement in the biopharmaceutical industry using survey tools (**36**), but no quantitative research has been published yet. Our research presents a comprehensive, data-based analysis of skills and domain knowledge related to biomanufacturing 4.0 in the United States.

Alternative studies that assess manufacturing skills gaps — typically related to "blue-collar" roles (**37–41**) — implement web-scraper technologies to collect data from labor-force websites such as LinkedIn, which provide impartial data on the general job market. Some other studies (**42**) use the "latent Dirichlet allocation" method for job-market data analysis. Such methods can help to identify expertise essential for jobs using data science and smart manufacturing, which are in great demand for today's manufacturing industry.

Our current investigation was stimulated by noted limitations in the existing literature on general manufacturing skills gaps. Surveys and interviews typically are limited in structure, with predefined questions that provide a complete representation of the labor market. Published studies predominantly focus on the demand for data, discounting the skillsets offered by the current and future workforces. Additionally, we've found a deficit in skills-gap research covering aspects of biopharma 4.0, including automation and data science.

Purpose

Below, we begin by describing the biopharmaceutical industry's transformative shift with the advent of industry 4.0 principles. We introduce existing key skills in the sector and the emergence of alternative skills that are required to harness the emergence of automation and digitization. And we hypothesize the skills gap as a critical challenge for which the complexities of biopharma 4.0 must be navigated to provide solutions.

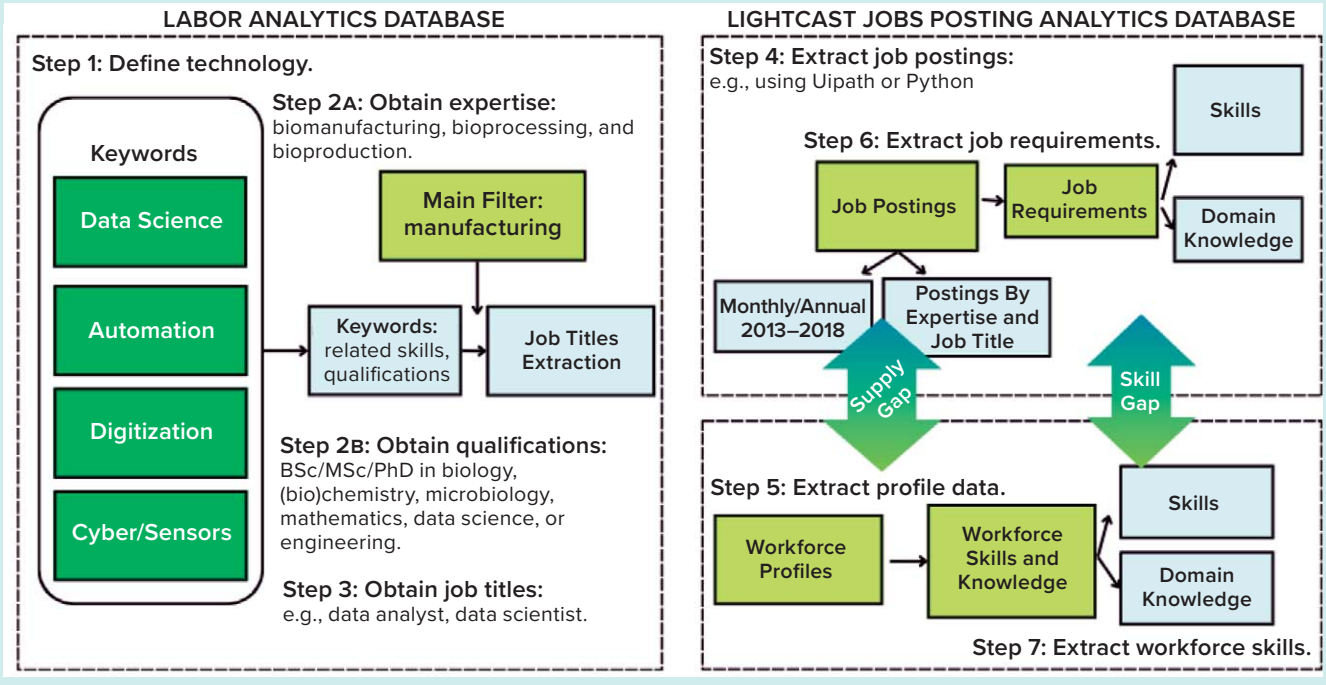
The next section illustrates methods we used to determine skills gaps, primarily applying programming interfaces to the Lightcast and LinkedIn databases to categorize data by job-posting keywords. We classified and organized profile and job-posting data collected over five years, and we assessed skills development over 20 years. Data collection is ongoing to assemble up-to-date expertise, skills, and domain knowledge requirements from the Lightcast Jobs Posting Analytics (JPA) module and professional profiles data from the Lightcast Profile Data (PD) module. In addition, we've supplemented those data with information from LinkedIn and university databases.

Beginning here in Part 1 and concluding in Part 2, our "Results" section first provides a summary of the skills/expertise gap at macro (technology clusters) and micro (specific expertise) levels, outlining the evolution of supply and demand relevant to skills needed in the biopharmaceutical industry. The relationship between talent and job frequency in different technology clusters highlights the availability and demand for specific skills. Part 2 also includes discussion and conclusion sections to elucidate current, emerging, and future trends within biomanufacturing skills and expertise, with a summary of our research findings and their limitations.

Methodology

Here we present our methodology for identifying the disparity between the skills and expertise required by biomanufacturing-sector job postings and the relevant skills and domain knowledge reported in professional profiles by job seekers. The raw data for this study came from the JPA and PD modules of the Lightcast labor-analytics database, together with triangulation information from LinkedIn and university databases. Because no previous quantitative biomanufacturing sector analysis has been executed, we adapted and improved on an analysis framework used in the automotive industry (**3**). This methodology is preferred over alternative skills-gap analysis methodologies for content analysis on job-market data. Although

Figure 1: Data collection and processing scheme for assessing the biopharma 4.0 skills gap

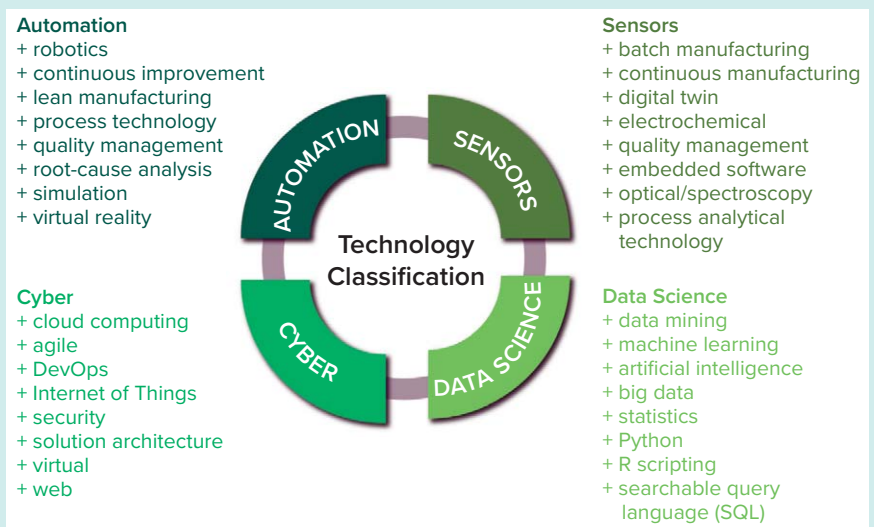


such data-driven methods can help the industry to recognize experience required for data science and smart-manufacturing–related jobs that are in high demand, the many disadvantages of such approaches include limited accuracy, difficulties in historical data acquisition, and a hindered ability to depict correlations and limitations in the topics generated.

To determine potential skills gaps resulting from the demands of industry 4.0, we used the Lightcast labor-analytics database to identify real-time labor-market patterns as well as the occurrence rate for particular skill and expertise terminology. Applying different filters provided for a customized search. Additionally, we analyzed requirements for specific competences and sector expertise recorded in job postings. For example, biomanufacturing companies active in the data-science job market often require applicants to have experience in programming languages such as Python, structured query language (SQL), and/or R script in addition to ML knowledge.

We categorized results by job-posting keyword and profile data filtered, ranked, and organized by skills, job titles, regions, and timeframes for the relevant source industries. We collected data over a five-year period (2018–2023) and assessed skills development over 20 years (2003–2023) with ongoing data collection for assembly of relevant expertise, domain knowledge, and skills requirements. As mentioned above, we used two Lightcast jobs modules supplemented by LinkedIn and university databases. Categorized data-extraction filters enabled the design of a chronological data-acquisition scheme (Figure 1).

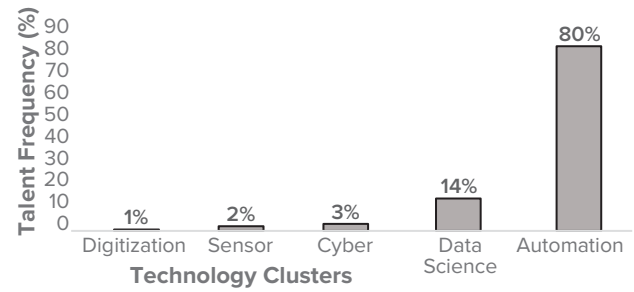
Figure 2: Technology classification and keywords for Lightcast database searches



Step 1 — Outline Technology Segments: The fourth industrial revolution (industry 4.0) originated in 2011 from a project in the high-tech strategy remit of the German government (**43**). It advanced the concept of cyberphysical systems (CPS) and "smart factories," the main associated initiatives of industry 4.0. With the advent of the Internet of Things (IoT), automation, and AI, connectivity between machines became the focus of industry 4.0. By contrast, industry 5.0 will prioritize improved collaboration between humans and machines through CPS and related technologies (**5**).

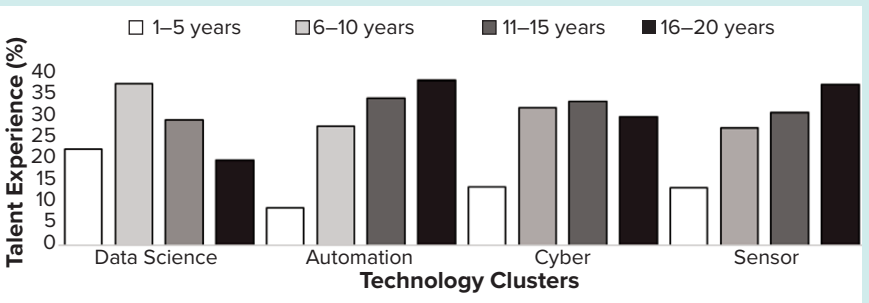
The most important constituent technologies of biopharma 4.0 are big data, the IoT, advanced processing technologies, robotics, augmented reality, simulations, CPS, cloud computing, and cybersecurity (**3**). As biopharma 4.0 evolves, AI is becoming

Figure 3: USA biomanufacturing talent frequency (%) by biopharmaceutical technology cluster (2023)



As biopharma 4.0 evolves, artificial intelligence is becoming more prevalent in bioprocessing to improve reliability, flexibility, and productivity in automation and big data exchange. However, smart manufacturing would not be possible without SENSORS.

Figure 4: USA biomanufacturing talent experience (%) by biopharmaceutical technology cluster (2000–2023)



more prevalent in bioprocessing to improve reliability, flexibility, and productivity in automation and big data exchange. And smart manufacturing would not be possible without sensors (**44**). So we segmented all these technologies into the clusters of *automation, digitization, data science, cyber,* and *sensors* — as depicted in Figure 2 — based on our own expertise and judgment.

Step 2 — Obtain Expertise: For each cluster, we applied the keywords of *biomanufacturing, bioproduction,* and *bioprocess* to generate data related to expertise and skills. Then we cross-reference those data in an additional industry 4.0 analysis to compare against the broader industrial sector by selecting codes 31–33 of the North American Industry Classification System (NAICS). The applied filters ensured that data gathered came from biomanufacturing companies and the biopharmaceutical industry's talent pool.

Table 1 outlines examples of biopharma 4.0 skills and expertise acquired from keyword searches. For each biomanufacturing segment, we identified particular keywords to obtain skills and expertise from the Lightcast database cross-referenced with data from LinkedIn:

- Data-science keywords include *data science, data mining, big data, AI, ML,* and *mathematical and statistical skills* (**3**)
- Automation keywords include *automation, virtual reality, robotics, processing technology, lean manufacturing, simulation, root-cause analysis, quality management,* and *continuous bioprocessing* (**2**)
- Cyber keywords include *security, agile, cloud computing, IoT, virtual, web, solution architecture,* and *DevOps (software development and operations)* — skills predominantly revolving around the IoT, cloud computing, and cybersecurity (**45**)
- Sensor keywords include *process analytical technology (PAT), embedded software, digital twin, spectroscopy, electrochemical, optical,* and both *continuous* and *batch manufacturing* — skills typically associated with signal processing and sensor analytics (**3**).

Step 3 — Job Description(s) Evaluation: We collected job titles, including segment-specific skills from Step 2, and summarized them in Table 2. No titles that were presented as "<×10" in the database (2018–2023) were included in this analysis.

Steps 4 and 5 — Job Postings and Profile Analysis: We analyzed job postings to conduct a gap analysis between the expertise availability (supply) and skills required (demand). Data came from 2018–2023 at both national (United States) and international (Europe, South Korea, and China) levels, as expanded upon in the Results section below and in Part 2. In addition, we also considered the geographical distribution of job postings and required educational qualifications.

Steps 6 and 7 — Job Requirements and Expertise Analysis: Expertise and skill requirements obtained included the aggregate number of job postings, significant profiles, and the most frequent skills based on their occurrence in each category. We performed a gap analysis by assessing the frequency of specific skills in the demand and supply data (see Part 2), which identified the biopharma 4.0 skills gap.

Results

As described above, we compared the talent supply semiquantitatively with talent demand at both the macro (by technology cluster) and micro (by defined expertise) levels. Cross-referencing Lightcast database information with data from LinkedIn, we compared the biomanufacturing talent frequency across the automation, digitization, data science, cyber, and sensor technology clusters of biopharma 4.0. Together, those specific segments generally cover the key talent involved in biomanufacturing sector supported with evidence of biopharma 4.0 adoption. The talent frequency can be defined as the "available talent pool" or "2023 talent supply," which can be compared against job postings using similar search criteria for "talent demand." To assess identified biopharma 4.0 skills deficits, we analyzed job postings and talent profiles

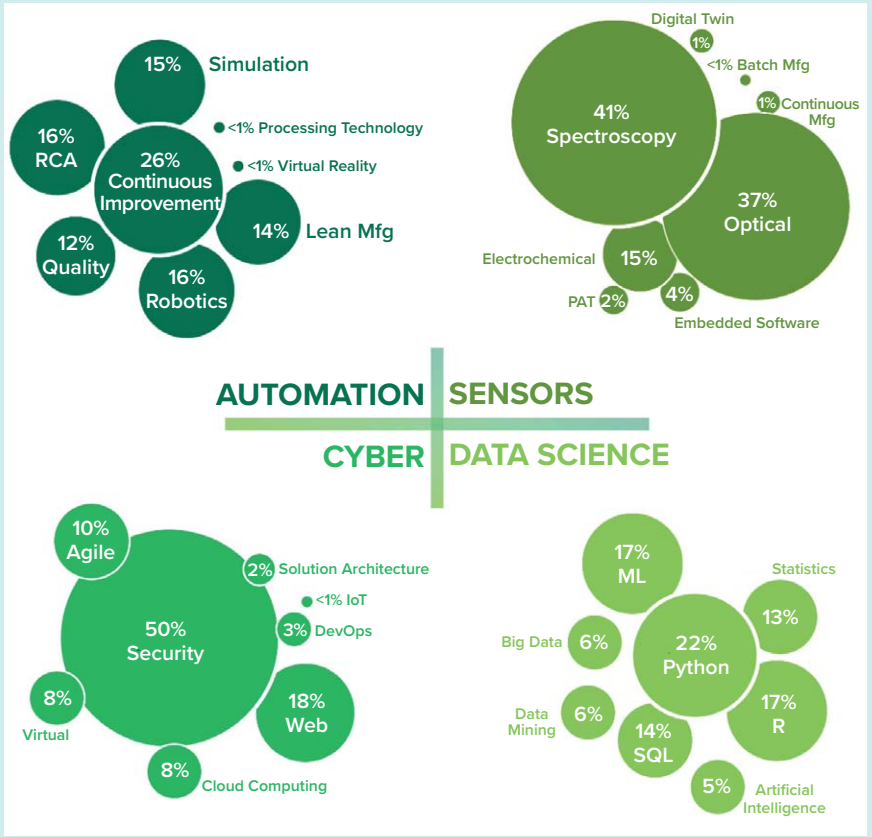
to conduct a gap analysis between expertise availability (supply) and skills required (demand) from data collected at both national (United States) and international (from Europe, South Korea, and China) levels from 2018 to 2023, taking the geographical distribution of job postings and required educational qualifications into consideration. Only USA data are presented herein.

Talent Supply: In cross-referencing Lightcast database information with data from LinkedIn, we compared the biomanufacturing talent frequency across the biopharma 4.0 technology clusters (technology segments) identified above. We chose those subdivisions to cover key talent involved in the biomanufacturing sector generally, supported by evidence of biopharma 4.0 adoption. We defined the talent frequency based on the available talent pool or "2023 talent supply" and compared that against job postings using similar search criteria for talent demand. These biomanufacturing sector divisions do not reflect a universal categorization.

Our aim was to represent biopharma 4.0 themes at a macro level and thus identify and collate the talent supply from biomanufacturing companies, illustrating evidence of technology adoption as defined by the boundaries of biopharma 4.0. Figure 3 segments the frequency of biomanufacturing talent in the United States based on the biopharma 4.0 technology clusters. Note that automation has a significantly higher frequency (80%) than any other cluster, suggesting that the available biomanufacturing talent currently has more evident skills in automation than in the data science, cyber, sensor, and digitization areas.

To explore the evolution of talent/expertise availability, we analyzed two decades of historic talent frequency data using the Lightcast database and cross-referencing with information from LinkedIn across the identified biopharma 4.0 technology clusters. Figure 4 clarifies that talent has matured through experience with automation — defined as an average 10–20 years of expertise per applicant (from the most recent qualification) — compared with other technology clusters with developing expertise. The cyber and sensors clusters seem to be relatively immature in expertise adoption, with only 1–5 years of experience per applicant from the most recent qualification. Note that data science is becoming more advanced, following automation, with average experience levels of 10 years from the most recent qualification. Results suggest that automation experience has evolved in correspondence with industry "on-the-job" education as biopharmaceutical technology platforms have been introduced and developed.

Figure 5: Talent supply by employee task application (micro level); DevOps = software development and operations, PAT = process analytical technology, RCA = root-cause analysis, SQL = searchable query language



For each technology cluster, we used certain keywords to collect skills, experience, and expertise from the Lightcast database, cross-referenced with LinkedIn data. Table 1 outlines example skills and expertise acquired from our keyword searches, and Figure 5 summarizes the talent supply at a micro level, referring to the biomanufacturing employee task application.

Automation: This technology segment includes skills and qualifications containing the keywords of *automation, virtual reality, robotics, processing technology, lean manufacturing, simulation, root cause analysis, quality management,* and *continuous bioprocessing (CB).* The majority of the available automation talent experience (26%) is focused on CB, which is the integration of multiple unit operations in biomanufacturing into a continuous, uninterrupted process flow (Figure 5).

CB provides several advantages over traditional batch processing, including increased productivity, reduced costs, improved process control, and enhanced product quality. Implementation of CB requires a skilled workforce with specific skills to operate and optimize these advanced manufacturing systems effectively. CB skills can be further segmented into process automation and control, in which people have expertise in programming, operating process control systems, and designing control strategies. Process monitoring and analytics generates a vast amount of real-time data, including process parameters, quality attributes, and sensor measurements. Technology

integration incorporates cell culture, separation, and purification processes into a seamless process flow. Quality by design (QbD), troubleshooting, process optimization, and regulatory compliance all require a strong understanding of regulatory requirements as well as knowledge of GMPs and the ability to design processes that meet regulatory expectations and ensure product safety and efficacy (**46**).

Note that the current lack of automation experience detected was specific to virtual reality (VR). While it has significant potential in the field of biomanufacturing, VR adoption and integration are in their early stages in the biopharmaceutical industry. However, as technology advances and VR becomes more accessible and affordable, its role is likely to expand, bringing about further advancements in process optimization, training, and research.

Data Science: This technology segment includes *data science, data mining, big data, artificial intelligence, ML,* and *mathematical and statistical skills*. Most data-science talent experience (22%) is in programming, with Python being the most common skill mentioned on LinkedIn profiles for the cluster, followed closely by R and ML (Figure 5). Python's ease of use, extensive libraries, and active user community make it a valuable tool in biomanufacturing. Its flexibility and integration capabilities enable scientists and engineers to streamline processes, analyze data, and develop advanced models and algorithms. As biomanufacturing continues to evolve, Python is likely to play a more significant role in areas such as data analysis and visualization, process modeling and simulation, automation and control, ML and AI, laboratory automation, web applications, and data sharing.

Figure 5 shows strong automation expertise (specifically CB) in the current biomanufacturing talent field. Data-science skills include evidence of Python programming in addition to expertise in cybersecurity and optical and spectroscopy sensors. However, note the lack of detected experience with VR, AI, and digital twins, particularly a low 5% of experience in AI. With the growing rate of AI adoption in biomanufacturing, professionals who possess AI skills can bring valuable insights and contribute to process optimization and data analysis. Such skills include working with ML, data analytics, statistical modeling, and programming languages (Python and R, particularly). AI skills enable individuals to analyze large data sets, develop predictive models, implement data-driven process optimization, and extract meaningful insights from complex biomanufacturing data.

Integration of AI in biomanufacturing is likely to grow, with talent contributing to the development of smart manufacturing strategies, the implementation of real-time monitoring and control systems, and the advancement of precision techniques throughout the sector. As the intersection between biomanufacturing and AI continues to progress, individuals with a combined skill set will be highly sought after in the industry (**46**).

Cyber: The cyber segment includes *security, cloud computing, agile, IoT, virtual, web, solution architecture,* and *DevOps*. Such skills primarily focus on cybersecurity, IoT, and cloud computing. The largest share of cyber expertise (50%) detected among biomanufacturing employees is specific to security (Figure 5). Biomanufacturing makes cybersecurity demands for ensuring the integrity, confidentiality, and availability of sensitive data and critical processes. Challenges include intellectual property (IP) protection, data security, supply chain security, operational technology (OT) security, and regulatory compliance. OT security poses a major challenge because biomanufacturing facilities rely heavily on interconnected OT systems, including process control systems, industrial control systems, and supervisory control and data acquisition (SCADA) systems. Such equipment must be protected from cyberthreats such as malware, unauthorized access, and manipulation. Implementing network segmentation, intrusion-detection systems, and regular security assessments can mitigate potential risks (**23**).

Sensors: The sensor segment includes keywords such as *PAT, embedded software, digital twin, spectroscopy, electrochemical, optical, CB,* and *batch manufacturing*. These skills relate mostly to signal processing and sensor analytics. The expertise detected for optical and spectroscopy sensors is high (37% and 41%, respectively) in Figure 5.

Optical and spectroscopy sensors play a crucial role in biomanufacturing processes and use light-based techniques to analyze samples, monitor key process parameters, and ensure product quality. Common applications of optical and spectroscopy sensors in biomanufacturing include those for biomass and cell-density monitoring, pH and dissolved oxygen (DO) monitoring, metabolite and product analysis, bioreactor imaging, and PAT. The use of optical and spectroscopy sensors enables continuous monitoring, process optimization, and quality control while providing valuable insights into critical parameters that facilitate data-driven decision-making and improve process efficiency and product quality (**23**).

We were surprised to see minimal experience with digital twins, which are virtual replicas or simulations of physical systems or processes based on real-time data from biomanufacturing sensors. By comparing real-time data with expected values and process models, a digital twin can help operators identify deviations, anomalies, and other potential issues. That allows for proactive intervention and control strategies to maintain process integrity, optimize process parameters, and ensure product quality (**47**). Integration of such technologies can facilitate the advancement of industry 4.0 principles in biomanufacturing, where data-driven insights and digital simulations improve the robustness and sustainability of production processes. The relative absence of talent in this area possibly relates to the evolving adoption of digital twins and required expertise — such as that with ML and predictive analytics — that require proficiency in ML algorithms and statistical modeling, within a digital-twin framework.

Looking Ahead

Part 2 concludes this report in BPI's

March 2024 issue by first shifting from a focus on supply to that on demand, then discussing emerging, current, and future trends. Finally, we summarize our research findings and elucidate their limitations.

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Continued from ***page 14***

critical staffing factor involved is finding experienced technical and production workers. The inability to hire for such roles was noted by 35.0% of industry respondents to our survey. Continued growth in capacity demand at new and existing facilities makes it particularly difficult to train and retain experienced manufacturing staff. Experienced workers sometimes are leaving regions with established biomanufacturing infrastructure for facilities in Asia and regions with emerging capabilities. Rapid growth in the CGT sector and industry responses to the COVID-19 pandemic also have created challenges over the past three years, complicating companies' efforts to retain stable workforces.

The Continuing Rise of Outsourcing: Over the past 17 years, the percentage of biotherapeutic developers performing all of their production activities in house clearly has trended downward. Meanwhile, the percentage of developers outsourcing such work to CMOs continues to rise.

Fewer companies are performing all of their manufacturing in house. That report aligns with a global trend that we have observed since 2006. The trend reflects the value of external service providers, which have become crucial to the industry. CMOs provide access to production capabilities, flexibility, and staffing that would be nearly impossible for some developers — especially small innovator companies — to establish internally.

A History of Resilience

Both current and historical data support a gradual return to prepandemic growth trends in production-capacity demand and consumables order volumes from suppliers. However, some business analysts are warning about a potential "COVID back-swing," during which facilities' inventory-management systems might not align with actual use. If that occurs, another period of transient shortages and delayed shipments could follow.

Nevertheless, the biomanufacturing industry's rebound from the unprecedented challenges left by the pandemic has showcased its resilience and ability to anticipate future needs. Although subsegments such as that for SU systems continue to negotiate near-term hurdles, the broader biomanufacturing segment has adjusted in ways that demonstrate innovative management and a rich history of adaptation and growth.

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Characterizing Oxygen Mass Transfer and Shear During Cell Culture

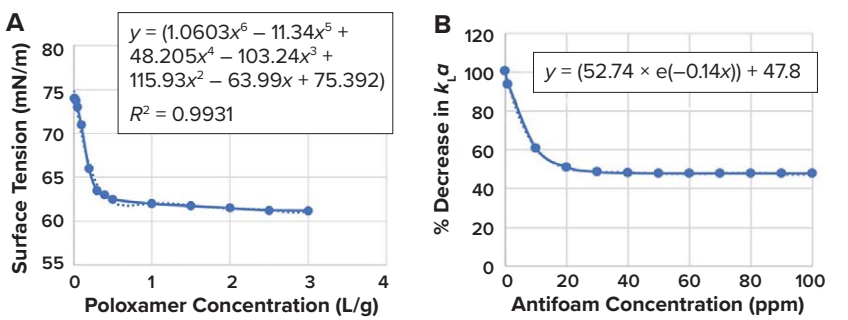
Calculating the Maximum Cell Density Supported By a 20,000-Liter Stirred-Tank Bioreactor

Naveenganesh Muralidharan, Emma Bolduc, and Mark Davis

Increasing demand for biologics and continuing limitations with single-use bioreactors (up to 5,000 L) compel some biopharmaceutical manufacturers to transfer their production processes into large-scale (20,000-L) stainless-steel bioreactors. Over decades of advancement, the industry has developed high–cell-density (HCD) cell-culture processes.

When transferring an HCD cell culture from 2,000-L to 20,000-L production bioreactors, process engineers first must assess whether the larger bioreactors can meet the high oxygen demands required for high-density cultures. If so, the next consideration is whether the process requires aggressive impeller-agitation and gas-flow rates for oxygenation, both of which can generate high levels of shear. Using aggressive impeller and gas-flow strategies will compel engineers to explore scale-up methodologies to minimize adverse impacts from excessive shear on cell growth and productivity.

Figure 1: (A) Effect of poloxamer 188 concentration on culture-medium surface tension; (B) effect of antifoam-agent concentration on bioreactor volumetric oxygen-transfer coefficient (k_La)



During cell-culture operations, oxygen is a key rate-limiting factor for cell growth and protein production. Cells consume dissolved oxygen (DO) in liquid media. Thus, the primary function of a bioreactor is to support cell respiration by maintaining needed DO concentrations, which can be accomplished through sparging and impeller agitation. Impeller agitation disperses sparged gas bubbles and promotes mass transfer of those bubbles through the gas–liquid (medium) interface.

The rate of oxygen transfer (OTR) from gas to liquid in a cell culture is a function of growth-medium properties and bioreactor design and operating parameters (**1, 2**). Herein, we present a study on OTR performed using a 20,000-L stirred-tank bioreactor (STBR). We discuss how data from such studies can be used to assess the maximum cell density that a given bioreactor can support. In addition, we elucidate a shear-proof design space for our bioreactor's operating conditions.

PRODUCT FOCUS: BIOLOGICS FROM MAMMALIAN CELL LINES

PROCESS FOCUS: PRODUCTION

AUDIENCE: MANUFACTURING AND PROCESS DEVELOPMENT

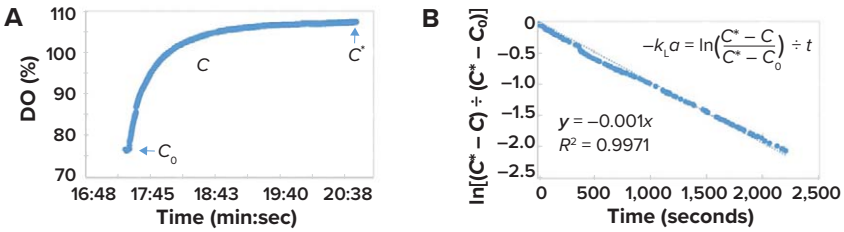
KEYWORDS: PREDICTIVE K_LA MODELING, STIRRED-TANK BIOREACTORS, IMPELLER AGITATION RATE, GAS-FLOW RATE, SHEAR

LEVEL: ADVANCED

METHODS FOR STUDYING OXYGEN MASS TRANSFER

Pseudomedium Preparation and Mass-Transfer Measurement: The *volumetric oxygen-transfer coefficient* (k_La) serves as a quantifiable measure of how efficiently oxygen is transferred from a gas phase to a liquid medium. The variable k_L is the mass-transfer coefficient (m/h), and a is the interface area available for mass transfer per unit volume of liquid. Because the dynamics of sparged gas bubbles vary continuously inside a bioreactor, measuring k_L independently from a and vice versa is difficult. Thus, those terms are measured together and reported as k_La in units of (1/h) (**3**). Bioreactor operating parameters that influence k_La include operating volume, impeller-agitation rate, and gas-flow rate through a sparger and overlay (**4–6**).

Figure 2: Calculating a bioreactor's volumetric oxygen-transfer coefficient ($k_L a$); panel (A) depicts dissolved oxygen (DO) levels, with C referring to a concentration at a given time (t), C_0 as the DO concentration upon initial pressure increase, and C^* as the saturation concentration; panel (B) plots results from application of the "dynamic pressure model" (DPM) to obtain $-k_L a$ values (**20**).



Equations 1–3: Effect of culture-medium components on volumetric oxygen mass-transfer coefficient

Equation 1:
$$S\left(\frac{g}{L}\right) = \frac{310\left(\frac{mOsmol}{kg}\right) \times \frac{1\ Osmol}{1{,}000\ mOsmol} \times 1\frac{kg}{L}}{\frac{1}{58.4}\left(\frac{mol}{g}\right) \times 2\ species\ per\ mol\left(\frac{Osmol}{mol}\right)} = 9\frac{g}{L}$$

Equation 2:
$$\%\ Decrease\ in\ k_L a = 52.74\ e^{(-0.14 \times CAnt)} + 47.8$$

Equation 3:
$$k_L = \frac{2}{\sqrt{\pi}} \times \sqrt{D_L}\left(\frac{\rho \varepsilon_L}{\mu}\right)$$

Effect of Salinity on $k_L a$: The solubility of DO decreases as salinity increases. Osmolality is a surrogate measurement of salinity. Most cell-culture growth media are formulated to an osmolality of 290–320 mOsmol/kg (**4**). We prepared a pseudomedium using a simple NaCl solution to mimic the osmolality of cell-culture medium. When NaCl dissolves in water, it completely dissociates into Na^+ and Cl^-. Thus, for every mole of NaCl per liter of medium, the osmolality increases by 2 Osmol/L (**7**). The amount of NaCl in our pseudomedium is calculated as 9 g/L (Equation 1).

Effect of Surfactant Concentration on $k_L a$: Pluronic F-68 poloxamer 188 surfactant (from BASF) is among the most frequently used shear-protecting agents in cell-culture medium formulation. Such agents reduce surface tension at the gas–liquid (medium) interface. However, the resulting drop in liquid surface tension follows an exponential decay curve that plateaus after 1 g/L (Figure 1A). Therefore, most commercially available, off-the-shelf cell-culture media are formulated to a poloxamer concentration of 1 g/L. Higher concentrations have little effect on liquid surface tension to protect cells from shear (**8**). Sieblist et al. report a small-to-negligible impact on $k_L a$ for poloxamer 188 concentrations of 0.1–1.0 g/L (**9**). Because bubble appearance and size depend strongly on sparger type, the team notes that their experiments involved drilled-hole spargers. Given such information in available literature, we did not include surfactant when preparing our pseudomedium.

Effect of Antifoam Concentration on $k_L a$: Routledge reports that addition of an antifoam agent — e.g., simethicone — increases bubble coalescence in cell-culture medium, leading to a reduced mass-transfer area and thereby reducing the $k_L a$ value (**10**). McAndrew and Kauffman provide an empirically derived equation that predicts the effects of antifoam concentration on $k_L a$ values (**11**). When evaluating simethicone concentrations of 1 ppm to 100 ppm with antifoam C emulsion solution in a drilled-hole–sparger bioreactor system, the writers observed a decrease of up to 50% in $k_L a$ values beyond concentrations of 30 ppm (Figure 1B). (Equation 2 provides the expression for the percentage reduction in $k_L a$ according to the antifoam concentration.) Figure 1B shows that the reduction in $k_L a$ follows an exponential decay curve that plateaus for antifoam concentrations exceeding 30 ppm. Because the amount of antifoam used in cell-culture processes usually ranges from 0 to 30 ppm, and because the effect of antifoam concentration on $k_L a$ is predictable, we did not include such surfactant when preparing our pseudomedium.

Changes in Bioreactor-Fluid Density and Viscosity: Several factors influence the magnitude and rate of change in culture-medium viscosity and density. They include viable-cell density (VCD), culture duration, supplement concentrations in a formulated growth medium, feed additions, cell-metabolism rates, and base-titrant consumption rates. During culture, cells produce proteins, debris, metabolites, and growth factors, all of which increase the surrounding fluid density and viscosity. At the same time, cells also consume proteins, glucose, nutrients, and growth factors, reducing fluid density and viscosity (**12**). In fed-batch mammalian-cell culture, fluid viscosity can range from 0.85 to 1.05 mPa.s, and the density can range from 1,007 kg/m^3 to 1,015 kg/m^3 over a transition from a cell-free medium to a spent HCD-harvest medium (**12, 13**).

Effect of Fluid Density and Viscosity on $k_L a$: Equation 3 represents Higbie's penetration theory for gas diffusion from gas bubbles rising in liquid (**14**). Here, D_L represents the diffusion constant for oxygen, ϵ_L is the dissipation rate of turbulent kinetic energy per unit mass, μ denotes the liquid viscosity, and ρ is the liquid dynamic density. Equation 3 shows a proportional relationship between liquid density and the mass transfer coefficient (k_L) and an inverse relationship between liquid viscosity and k_L.

In accord with Higbie's penetration theory, Xu et al. and Solecki report that increasing viscosity results in a reduced $k_L a$ value (**15, 16**). Moreover, Koide et al. and Yoshida et al. write that an increase in liquid density results in a $k_L a$ increase (**17, 18**).

Magnitude of Change in Density and Viscosity During Cell Culture and Its Effect on $k_L a$: Pappenreiter et al. observe no significant changes in $k_L a$ between cell-free media and spent harvest media from a Chinese hamster ovary (CHO) fed-batch culture (**19**). Therefore, changes in viscosity are considered to have a minimal overall impact on such

Figure 3: Parameters for k_La assessment (lpm = liters per minute, rpm = revolutions per minute, and kL = kiloliters)

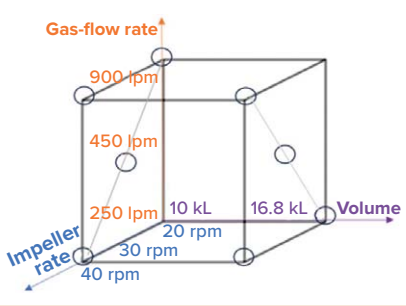
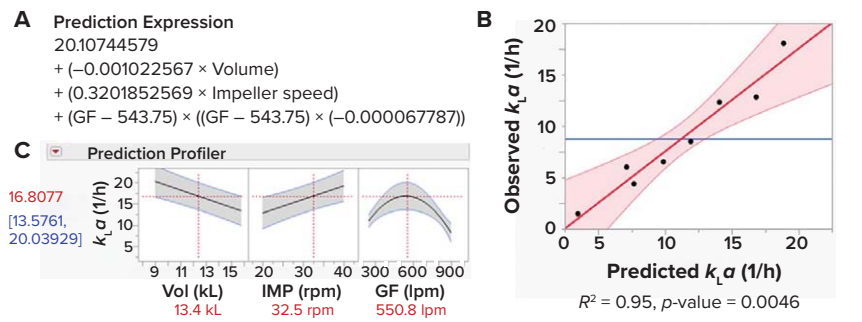


Figure 4: Predictive model for measuring a bioreactor's volumetric oxygen-transfer coefficient (k_La)



Equations 4–6: Oxygen transfer rate (OTR)

Equation 4: $OTR = k_La(C^* - C_L) = k_La(1.07\frac{mmol}{L} - 0.112\frac{mmol}{L}) = k_La \times 0.96\frac{mmol}{L}$

Equation 5: $C^* = H \times P \times f_{oxy} = 1.07\frac{mmol}{L.atm} \times 1\ atm \times 1 = 1.07\frac{mmol}{L}$

Equation 6: $C_L = H \times P \times 0.21 \times DO_{st.pt} = 1.07\frac{mmol}{L.atm} \times 1\ atm \times 0.21 \times 0.5 = 0.112\frac{mmol}{L}$

Figure 4C depicts a **PREDICTION PROFILER** for the magnitude of change in the response variable (k_La) that occurs when one input parameter is changed while all others are held constant.

values. Hence, we did not implement changes in viscosity and density for our k_La predictive-model study.

Measurement Method: Linek et al. propose a method for accurate measurement of k_La in a large-scale bioreactor, a concept that they call the "dynamic pressure method" (DPM) (**20**). In the DPM, when the measured DO value is stabilized for specific bioreactor operating parameters (such as culture volume, impeller agitation rate, sparger gas-flow rate, and pressure), then total pressure increases by 20%.

According to Henry's law, an increase in bioreactor pressure will lead to a simultaneous change in the oxygen concentration of all bubbles that are dispersed in the liquid, thereby reducing the influence of nonideal mixing of the gas phase on the k_La measurement. Upon initiation of a step-change increase in bioreactor pressure, the %DO value increases and reaches a new saturation value (Figure 2A). As shown in Figure 2B, plotting the expression $\ln[(C^* - C) \div (C^* - C_0)]$ as a function of time yields a slope of $(-k_La)$. For the equations in those figures, t is the time in seconds, C^* is the saturation concentration of oxygen (expressed as a percentage), C_0 is the concentration of oxygen (as a percentage) at initial pressure change, and C is the oxygen concentration at any time (as a percentage) (**5, 20**).

Predictive K_LA Modeling

Bioreactor Power: We applied a partial-factorial design form as the design of experiment (DoE) to characterize k_La and develop a predictive response-surface model. For k_La characterization, we selected the following inputs:

- impeller agitation rate — at 20, 30, and 40 revolutions per minute (rpm)
- sparger air-flow rate — at 250, 450, and 900 liters per minute (lpm)
- vessel working volume — at 10,000 L and 16,800 L.

We studied eight conditions with different combinations of volume, impeller agitation rate, and sparger gas flow rate (Figure 3). For each condition, we measured and calculated a k_La value using the DPM. Applying a standard least-square regression model (using JMP 16.0 software) enabled development of a predictive model, which is shown in Figure 4A.

Figure 4B displays observed and predicted values. R^2 is a statistical measure of how well a predictive expression fits observed data. The obtained value of R^2 = 0.95 demonstrates the reliability of our predictive expression. Moreover, we obtained a p-value (0.0046) <0.05, demonstrating that the strength of association between the input and output variables is statistically significant.

Figure 4C depicts a prediction profiler for the magnitude of change in the response variable (k_La) that occurs when one input parameter is changed while all others are held constant. As expected, Figure 4C shows increases in k_La and impeller-agitation rates with a decrease in working volume. Of interest is that our data show an increase in k_La with increasing gas-flow rate up to 550 lpm; however, k_La starts decreasing at higher flow rates. That result could be explained as an impeller flooding regime. At a certain combination of low impeller agitation speed and high gas-flow rate, bubbles are not dispersed across the liquid by impeller agitation and simply escape out of the liquid (**6**).

Calculating Maximum Cell Densities Using K_LA Values

Measuring Oxygen Mass Transfer: OTR measures how much oxygen can be transferred in a bioreactor per unit of time for a specific k_La value at a specified fraction of oxygen in the gas phase. That rate can be calculated using Equations 4–6 and k_La values (1/h) determined by the predictive-model expression shown in Figure 4A.

As noted above, C^* is the saturation concentration of oxygen in mmol/L, as

Equations 7–8: Oxygen transfer rate (OTR), oxygen uptake rate (OUR), and maximum cell density; Q_{O2} = specific oxygen-consumption rate for cells in a bioreactor, $k_L a$ = volumetric oxygen-transfer coefficient

Equation 7: $OUR = x_i \times Q_{O2}$

$$= \frac{10^6\ cells}{mL} \times 10^3\ \frac{mL}{1\ L} \times 5.5\ \frac{10^{-12}\ mol}{cell.day} \times \frac{10^3\ mmol}{mol} \times \frac{1\ day}{24\ h} = 0.23\ \frac{mmol}{L.h}$$

Equation 8: Maximum cell density:

$$x_{i,max}\left(\frac{10^6\ cells}{mL}\right) = \frac{k_L a \frac{1}{h} \times 0.96\ \frac{mmol}{L}}{0.23\left(\frac{mmol}{L.h}\right)} = k_L a \times 4.18$$

Equations 9–10: Dynamic change in specific oxygen uptake rate

Equation 9: Predicted viable-cell density: $x_n = x_{n-1} \times e^{\Delta t \times \mu}e$

$\Delta t = t_n - t_{n-1}$: duration (in hours) μ : assumed specific growth rate (1/h)

x_n : cell density at t_n x_{n-1} : cell density at t_{n-1}

Equation 10: Specific oxygen-uptake rate: $Q_{O2} = 17.328 \times \mu^{0.2813}$

μ : assumed specific growth rate (1/h)

calculated from Equation 4. The variable H denotes Henry's coefficient for oxygen solubility in water. For typical culture operating conditions at 37 °C, H = 1.07 mmol/(L.atm) is a good approximation.

The average bioreactor pressure (in atmospheres) is denoted as P. In a bioreactor, positive pressure is maintained; pressure in the headspace and from the liquid height sum up to be >1 atm. However, for simplicity in our calculation, we assume that P = 1 atm.

The variable f_{oxy} represents the fraction of oxygen in the incoming gas. The fraction of oxygen in air is 0.21 (21%); however, to assess the maximum capability of our bioreactor, we assume here that it is sparged with pure oxygen (f_{oxy} = 1). The variable C_L is the setpoint oxygen concentration maintained in the bioreactor (expressed in mmol/L), as calculated from Equation 6. DO probes are calibrated to 100% through exposure to atmospheric oxygen concentrations. Therefore, a 50% DO setpoint indicates 50% of 21%.

Measuring Oxygen Uptake: Oxygen is crucial to cell metabolism. The primary metric for bioreactor capability is the system's ability to meet cells' oxygen-respiration rate — the *oxygen uptake rate* (OUR). As shown in Equation 7, the OUR is the product of the *VCD* value and the specific oxygen-consumption rate of cells in a bioreactor (Q_{02}) (**4**). The latter variable is defined as the amount of oxygen consumed per cell per unit time (pmol/(cell.day)). The variable X_i represents the VCD (10^6 cells/mL).

Specific OUR (Q_{02}) Values Differ Across Mammalian Cell Lines: For mammalian cells, Q_{02} values are cell-line specific. Jorjani et al. report maximum Q_{02} values for baby hamster kidney (BHK), murine hybridoma, and CHO cells that are cultured at 37 °C as 7.2, 5.28, and 7.44 pmol/cell.day, respectively (**21**).

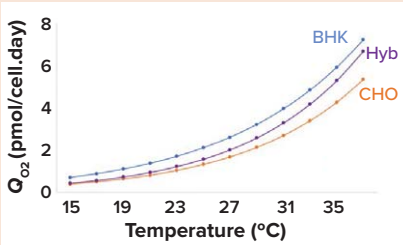
Effect of Temperature on Q_{02} Values for Mammalian Cells: During cell-culture processes, operators often shift temperature from 37 °C to 29–35 °C to increase productivity and maintain product quality. Such a downward temperature shift has been shown to improve cell viability and specific productivity (**22, 23**). All biological and chemical reactions are temperature dependent and can be modeled by the Arrhenius equation (see Figure 5). There, A is the Arrhenius constant, and E represents the activation energy (kJ/mol). R is the ideal-gas constant (0.00831 kJ/mol.K), and T is the temperature in kelvins. Figure 5 models the change in Q_{02} as a function of temperature for BHK, hybridoma, and CHO cell lines using A and E values reported by Jorjani et al. (**21**).

Dynamic Change in Bioreactor Q_{02} Values: The change in cell density per unit of time is defined as the growth rate (cells/h); the change in cell density per cell per unit of time is defined as the specific cell-growth rate (cells/(cell.h)) or (1/h). In protein-biologics manufacturing, bioreactors often are operated in fed-batch mode. As cells proliferate during culture, the medium composition changes constantly, diminishing nutrient levels and accumulating toxic metabolites. That situation changes the cells' physiological state and thus reduces the specific cell growth rate.

The VCD profile of a culture in batch or fed-batch mode includes phases for exponential cell growth, stationary growth, and decline. Cell growth rates begin high (during the exponential phase) and plateau as cells enter the stationary phase. Growth rates fall as cells enter the declining phase. Note that the specific cell growth rate represents cells' physiological state (and thus their ability to proliferate) rather than their growth rate as such. In fed-batch cell cultures, analysts typically observe both a decrease in the specific cell-growth rate and an increase in the rate of cells produced.

Effect of Culture Time on Cell-Specific OUR: Ihling et al. report that the specific OUR of a CHO-cell culture decreased

Figure 5: Specific oxygen-uptake rate (Q_{02}) as a function of temperature for baby hamster kidney (BHK, blue), murine hybridoma (Hyb, purple), and Chinese hamster ovary (CHO, orange) cell lines



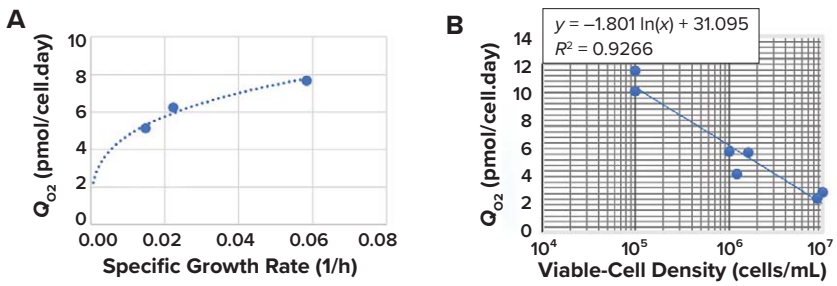
$$Q_{O2}\ \frac{pmol}{cell.day} = (A.e^{-E/R.T}) \times 24$$

Table 1: Values for calculation of specific oxygen-uptake rate (Q_{02}) in Figure 5 for baby hamster kidney (BHK), murine hybridoma, and Chinese hamster ovary (CHO) cell lines; A = Arrhenius constant, E = activation energy, and R = gas constant

Cell Line	A Value	*E* Value
BHK	6.08×10^{12}	79 kJ/mol
Hybridoma	3.20×10^{14}	90 kJ/mol
CHO	1.28×10^{15}	93 kJ/mol

R = 0.008315 kJ/mol.K

Figure 6: Comparing specific oxygen consumption rate (Q_{O2}) with specific growth rate and viable-cell density (VCD)



Equations 11–13: Impeller mixing time

Equation 11: Impeller discharge rate (m^3/s): $Q_{imp} = N_F \times n \times d_i^3$

Equation 12: Impeller circulation time (s): $t_{cir} = \frac{V}{Q_{imp}}$

Equation 13: Impeller mixing time (s): $t_{mix} = 4 \times t_{cir}$

Figure 7: Typical values for cell growth rate, specific cell growth rate, and specific oxygen consumption rate (Q_{O2}); data provided by AGC Biologics.

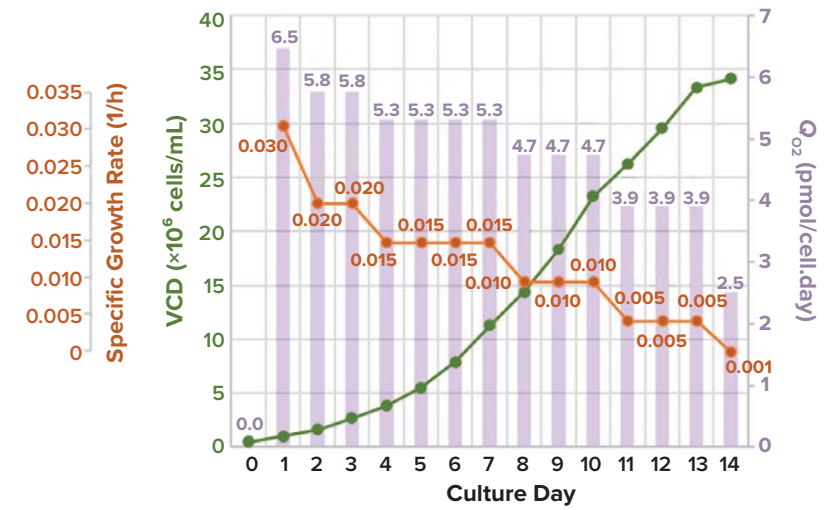
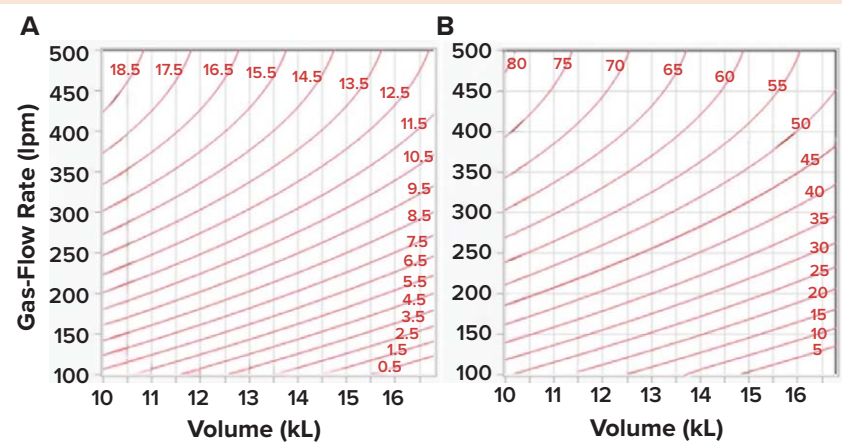


Figure 8: (A) Contour profile showing k_La values (1/h) obtained using the prediction expression in Figure 4A; values were determined as a function of volume (calculated in liters, listed below in kiloliters) and gas-flow rate (in liters per minute, lpm) for an impeller-agitation rate of 30 rpm. Panel (B) presents a contour profile of the maximum viable-cell density (VCD, 10^6 cells/mL) predicted by Equation 9 for each k_La value in Figure 8A.



from 5.7 to 2.4 pmol/cell.day at 96 hours after inoculation (**24**). Deshpande et al. similarly observe a specific OUR decrease from 7.7 to 4.8 pmol/cell.day as early as 50 hours after inoculation (**25**).

Effect of Specific Growth Rate on Cell-Specific OUR: As cells proliferate, they require more oxygen for respiration. Thus, specific OURs are directly proportional to specific growth rates (**3**). Using data reported by Deshpande et al. (**25**), we developed an exponential correlation expression between the cell specific growth rate and specific OUR (Figure 6A) (Equation 10).

Effect of Cell Density on Cell-Specific OUR: As VCD increases during culture, so do metabolite concentrations because of cell metabolism and feed additions. Those increases reduce specific OURs. Sand et al. identify that phenomenon as a "metabolic-crowding effect" in mammalian suspension cell culture (**26**). As shown in Figure 6A, we developed a logarithmic correlation expression between VCD and specific OUR based on data from Wohlpart et al. (**27**) and Deshpande et al. (**25**). For a typical mammalian-cell culture in a fed-batch bioreactor operation with a cell density of 0.5–50 × 10^6 cells/mL, the specific oxygen consumption rate ranges from 6.0 to 3.5 mmol/cell (Figure 6B).

For the purpose of illustration, we assume a specific growth rate ranging from 0.03 to 0.001 (1/h) and a seeding density of 0.5 × 10^6 cells/mL (**28**), and using Equations 9 and 10, we simulated a VCD profile and corresponding cell-specific OUR (Figure 7). The results show that Q_{02} = 5.5 pmol/(cell.day) is a good approximation of conditions during typical mammalian-culture bioreactor operation. That expression can be applied for conservative calculation of maximum VCD when assessing bioreactor capability (1 pmol = 10^{-12} mol) (**29, 30**) (Figure 8).

During cell-culture operations, bioreactor OTR should equal bioreactor OUR. By setting Equations 4 and 7 as equal, we can calculate the maximum cell count that can be supported for a given k_La value. Equation 8 indicates that ~4 × 10^6 cells/mL can be supported for a k_La value of 1 (1/h) when pure oxygen is used as the sparge gas.

Figure 9: (A) Comparing mixing time (s) with impeller-agitation rate (rpm); (B) plotting tip speed (m/s) and power:volume ratios (P/V, in W/m^3) versus impeller-agitation rate

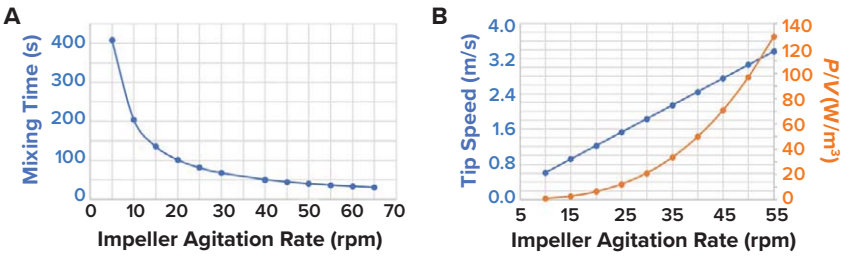
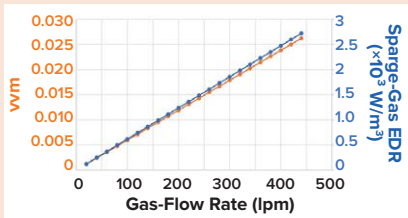


Figure 10: Volume of liquid per minute (vvm) and sparge-gas energy dissipation rate (EDR, in W/m^3) plotted against gas-sparge flow rate (in liters per minute, lpm)



Assessing Bioreactor Mixing Time

The *impeller discharge rate* (m^3/s) is the volume pumped from an impeller blade per unit of time. Analysts can calculate that rate using Equation 11, in which N_F represents the impeller flow number, n is the impeller agitation rate (in rpm), and d_i signifies the impeller diameter (in meters). *Circulation time* is defined as the ratio of liquid volume (in m^3) to impeller discharge rate (in m^3/s) (Equation 12). *Bioreactor mixing time* refers to the time required to combine completely segregated culture materials to a given level of homogeneity. After several circulations, a desired homogeneity is reached (**2**). Doran reports that the mixing time for a STBR with several baffles and a small impeller-to-tank-diameter ratio is about four times the circulation time (**2**) (Equation 13).

In our 20,000-L bioreactor, the agitation system includes two Lightnin A320 impellers (SPX Flow) with an N_F value of 0.62. Figure 9A plots mixing times for different impeller-agitation values. As shown there, we calculated that mixing time would not improve significantly with agitation rates beyond 30 rpm.

Assessing Shear and Impeller-Agitation Rates

Once we established a k_La predictive model, our next step was to identify limitations on impeller-agitation rates. The most frequently used parameters for scaling up impeller-agitation rates are the power-to-volume ratio (P/V, expressed in W/m^3) and impeller-tip speed (in m/s).

Power indicates energy consumed over time. Thus, P/V represents the average power consumed by an impeller divided by a bioreactor's entire volume. The P/V approach helps process engineers not only to scale impeller speeds for different sizes of a given impeller design, but also to scale between different impeller designs and geometries; impeller-power calculations accommodate design differences in impeller configurations (**31–33**). Commercial-scale bioreactors typically operate at P/V values of <50 W/m^3 to minimize deleterious effects on cell health (**35**). Equation 14 lists the calculation for P/V. There, N_p represents the impeller power number provided by the manufacturer, and ρ is the culture-medium density, for which 1,015 kg/m^3 is a good approximation (**12**).

Although average P/V is used across the biopharmaceutical industry as a parameter for determining impeller speeds for a range of STBR scales, the metric does not account for scale-dependent shear heterogeneity, which often occurs inside bioreactors and is especially prevalent in large-scale STBRs. Hu, Berdugo, and Chalmers observe that 70.5% of the total power consumed by a pitched-blade impeller dissipates in the liquid volume near the impeller zone (**36**). *Shear rate* is the change in fluid velocity over a given distance. In impeller-based STBRs, impeller-tip speed serves as a surrogate measurement for the maximum shear level inside the reactor because the maximum fluid velocity is observed at the tip of the impeller blade as it revolves in the fluid (**33**).

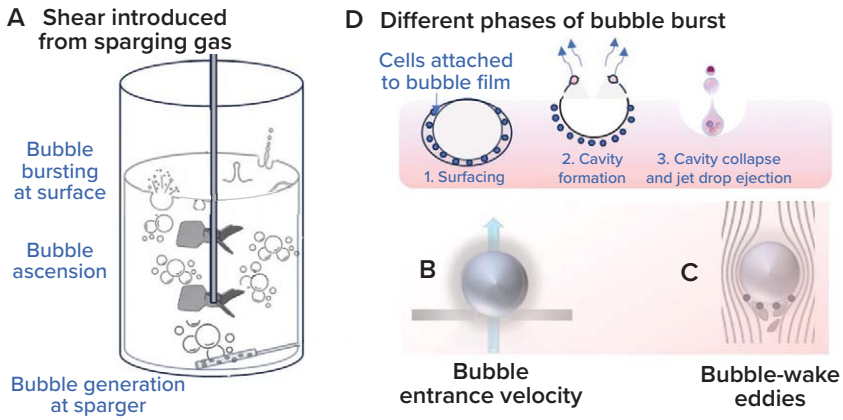
For a P/V value maintained across different sizes of bioreactors, impeller-tip velocity rises with increases in bioreactor scale (**4**). Hence, tip velocity is deemed to be a critical parameter needing evaluation when scaling impeller speed. Such assessments consider only the impeller diameter and agitation speed, not the impeller design.

Equations 14–15: Shear according to impeller-agitation rate

Equation 14: Power-to-volume ratio (W/m^3): $$\frac{P}{V} = \rho\left(\frac{N_p d_i^5 n^3}{V}\right)$$

Equation 15: Impeller-tip speed: $$\theta_{Imp,Tip} = \frac{\pi d_i n}{60}$$

Figure 11: Localized shear from gas sparging (adapted from reference **34**)



Equations 16–27: Shear from sparging gas

Shear When a Bubble Forms at a Sparger Hole — Bubble-Entrance Velocity

Equation 16: Average EDR from gas sparging = $\frac{Q_g \times P2}{(\pi d_T^2) \div 4} \times \frac{\ln(P1 \div P2)}{(P1 - P2)}$

$P2 = P1 + (\rho \times g \times h_T)$

Equation 17: Gas-entrance velocity = $Q_g \div \left(n_0 \times \frac{\pi d_T^2}{4}\right)$

Shear When a Bubble Rises Through the Bioreactor Liquid Height — Bubble-Rise Eddy Length (38)

Equation 18: Bubble area (m^2): $A_b = \frac{\pi d_b^2}{4}$

Equation 19: Bubble velocity (m/s): $\theta_b = \left(\frac{2\sigma}{d_b \rho} + \frac{0.5 d_b}{g}\right)^{1/2}$

σ : liquid surface tension (0.65 N/m)

ρ : liquid density (1,015 kg/m^3) g : acceleration due to gravity (9.8 m/s^2)

Equation 20: Drag force from bubble ($kg.m/s^{-2}$): $F_b = (C_d \times \theta_b^2 \times A_b \times \rho) \div 2$

C_d : drag coefficient for rising bubbles, assumed to be 2.6 (dimensionless) (**38, 47**)

Equation 21: Bubble EDR (W/m^3 or $kg.m^2/s^{-3}$): $F_b.\theta_b$

Equation 22: Specific EDR (W/kg): $\varepsilon_b = 0.8 \times (\text{EDR}) \div \left(\frac{\pi d_b^3}{6} m^3 \times 1{,}000 \frac{kg}{m^3}\right)$

Equation 23: Bubble-eddy length: $\eta b = 10^6 \left(\frac{v^3}{\varepsilon_b}\right)^{1/4}$

Shear When a Bubble Bursts at the Gas–Liquid Interface — Bubble-Burst Eddy Length

Equation 24: $\text{EDR}_{\text{burst}} = e^{-194.43 \times (\log(d_b))^3 - 217.02 \times (\log(d_b))^2 - 129.07 \times (\log(d_b)) - 19.599}$

Here, bubble diameter (d_b) is calculated in centimeters.

Cell Death Rate Constant

Equation 25: Rate of bubble generation: $R_b = (Q_g \div v_b) \div v = \frac{Q_g}{(\pi d_b^3) \div 6} \div v$

Equation 26: Kill volume: $V_{kill,b} = \frac{\pi d_b^3}{6} - \frac{\pi (d_b + \delta)^3}{6}$

δ : bubble-film thickness = 50 µm (**38**)

Equation 27: First-order bubble death rate constant: $K_{d,bub} = V_{kill,b} \times R_b$

Impeller-tip speed is calculated using Equation 15. For typical cell-culture processes, process engineers recommend maintaining an impeller-tip velocity of 0.6–1.8 m/s to prevent adverse effects on cell growth (**37**). Figure 9B plots the impeller-tip speed and P/V at different agitation rates for an STBR with an operating volume of 16,800 L. We observe a limitation in the impeller agitation rate at 30 rpm for a maximum acceptable impeller-tip velocity of 1.8 m/s and at 40 rpm based on the maximum acceptable P/V limit of 50 W/m^3. Therefore, we conservatively set 30 rpm as the impeller rate based on a maximum tip speed of 1.8 m/s.

Assessing Shear and Gas-Flow Rate

Key Parameters: Process engineers often assess the average amount of shear in an STBR using the energy-dissipation rate (EDR) for sparged reactors, as reported by Bhavaraju, Russell, and Blanch (**38**). The average EDR is calculated from Equation 16, in which Q_g is the gas-flow rate (in m^3/s), d_T is the bioreactor-tank diameter, g is the acceleration rate (9.8 m/s^2), h_T is the bioreactor liquid height (in meters), and $P1$ and $P2$ represent the pressures on the surface of the bioreactor liquid and at the sparging point, respectively (in $kg.m^{-1}.s^{-2}$ or pascals). Based on values from 24 studies of shear assessment, Hu, Berdugo, and Chalmers report an EDR of 10^4 W/m^3 as sublethal for most mammalian cells (**36**).

Another parameter that is assessed frequently when scaling cell-culture gas-flow rates is the gas volumetric rate per bioreactor unit volume, expressed as the volume of air per unit volume of liquid per minute (vvm). That parameter influences shear levels differently across bioreactor scales, and holding it constant does not account for differences in sparger configurations and designs, which often are distinctive to bioreactor models and manufacturers (Figure 10). Chisti reports a decrease in specific cell-growth rate at gas-flow rates >0.04 vvm (**39**).

Other parameters for scaling bioreactor gas-flow rates in STBRs include *superficial gas velocity*, which is a ratio of the volumetric gas-flow rate to a bioreactor's crosssectional area. Because the calculation for that parameter is part of the average EDR equation for gas sparging (Equation 16), superficial gas velocity needs no further discussion for our purposes.

Bubble Assessment: Localized shear introduced by sparging gas into an STBR is assessed at three phases: when a sparger hole generates a gas bubble, when that bubble rises upward across the liquid height in a bioreactor, and when the bubble reaches the surface and bursts at the gas–liquid surface interface (**39**) (Figure 11A). **When bubbles are generated**, shear can be assessed based on gas-entrance velocity (GEV) at the sparger orifice (Figure 11B). GEV is calculated as a ratio of the volumetric gas-flow rate to the total area available for gas to exit (Equation 17). In that calculation, Q_g denotes the gas-flow rate (in L/s), d_o signifies the diameter of a single sparger hole (in meters), and n_o is the number of holes in the sparger. Zhu et al. (**40**) and Chaudhary et al. (**41**) identify >30 m/s and >60 m/s as critical GEV values for preventing adverse effects on cell growth and productivity in nonsecreting murine myeloma (NSO) and CHO cells, respectively. Figure 12A shows that the critical GEV of 60 m/s for CHO cells is reached at a sparge gas-flow rate of 200 lpm.

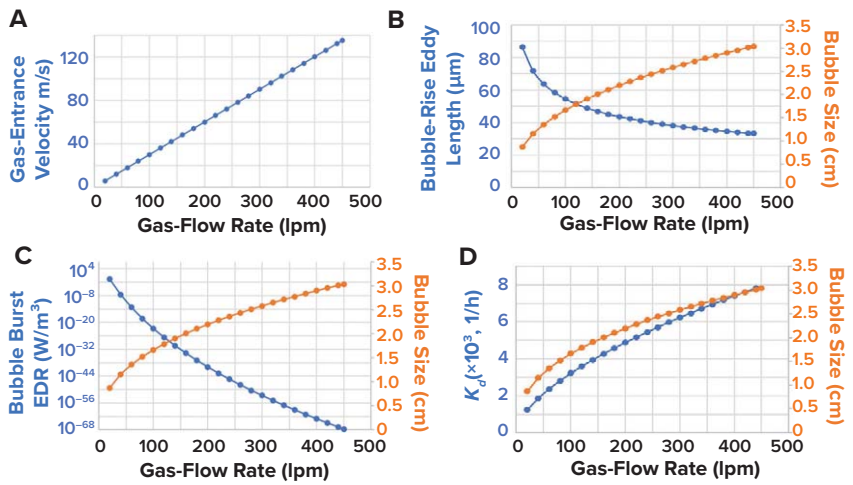
When evaluating **bubbles rising along the bioreactor liquid height**, shear levels

are based on bubble-wake microeddy length. As bubbles rise through a bioreactor, the circulating fluid carried behind them experiences the greatest drag force (Figure 11C). Bubble-wake eddy length can be calculated from Equations 18–23 (**39**). A microeddy length that is less than or equal to the diameter of cultured cells is detrimental to cell viability. Hence, that length should be maintained at >20 µm for most mammalian cell-culture operations (**4, 39**). Figure 12B shows that, for sparge gas-flow rates up to 450 lpm, the bubble wake eddy length is >34 µm.

As a bubble rises, cells become trapped in the film around it. A cavity forms on the bubble surface as it reaches the gas–liquid interface, and as the bubble bursts and the cavity collapses, the resulting liquid jet shears the cells carried in the bubble-film volume (Figure 11D). **Shear from bubble bursting** is assessed by calculating the EDR at the burst location as a function of bubble diameter (Equation 24). We derived Equation 24 from data presented by Boulton-Stone and Blake (**42**). That information was obtained using a nonlinear exponential model with JMP 16.0 software (**36, 42–44**). Walls et al. observe that CHO cells die when exposed to EDR levels >10^6–10^8 W/m^3 (**44**). Hu, Berdugo, and Chalmers report that EDR levels of ~10^4 W/m^3 are sublethal for most cell-culture operations (**36**). Figure 12C shows that the bubble-burst EDR is <10^4 W/m^3 across the entire range of applied gas-flow rates (20–450 lpm).

The above method for calculating bubble-burst EDR depends on the bubble diameter only; however, the technique does not account for the total amount of bubbles generated in a culture or the rate of bubble generation. Tramper et al. present a first-order calculation for the cell-specific death rate constant (K_d) as a function of bubble size, rate of bubble generation per unit volume of culture, and bubble film volume or kill volume (**45**). The latter parameter is the volume of media over which cells are carried before experiencing shear as a bubble bursts at the gas–liquid interface (**26**). The death rate constant can be calculated from Equations 25–27. For shear-proof control conditions, Tramper et al. (**45**) and Chisti (**39**) demonstrate that the death rate constant is ~0.032 (1/h). Thus, a gas-sparger design yielding a K_d value of <0.032 (1/h) helps to ensure shear-proof conditions for the cells. Figure 12D shows that, for sparge gas-flow rates up to 450 lpm, the bubble-burst EDR is <10^4 W/m^3, while k_d is <0.032 (1/h).

Figure 12: (A) Gas-entrance velocity (GEV, m/s) plotted against gas-flow rate (lpm); (B) bubble diameter (cm) and bubble-rise eddy length (µm) against gas-flow rate (lpm); (C) bubble diameter (cm) and bubble-burst energy dissipation rate (EDR, W/m^3) at the gas–liquid interface, compared with gas-flow rate; and (D) bubble diameter (cm) and sparging cell-death–rate constant (1/h) plotted against gas-flow rate



Determining Maximum Cell Density in an STBR

The presented assessment for determining the maximum cell density that can be supported for a given k_La value assumes 5.5 mmol/(cell.day). That value is appropriate for most fed-batch mammalian-cell cultures. However, Goudar et al. note that some clones of CHO cells have cell-specific OURs of <10 pg/(cell.day) (**30**). Process engineers and upstream-production scientists should evaluate the assumed specific OUR carefully in such cases. For shear assessment, the maximum tip speed, GEV, and bubble-burst EDR represent the region of a shear-proof design space based on previously reported studies. That region does not necessarily indicate the edge of failure; therefore, if necessary, a marginal increase in any of the above shear assessments could be explored with an appropriate scale-down model or detailed risk assessment.

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Streamline Manufacturing of Antibody-Based Therapeutics with Novel Purification Approaches

Laurens Sierkstra

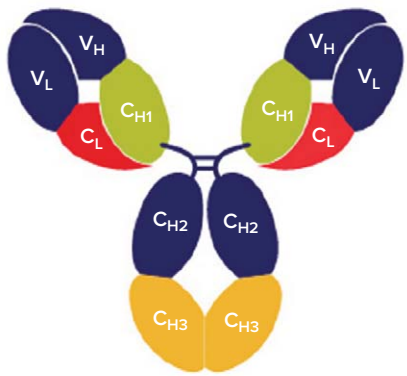
Using affinity capture in purification of antibodies enables developers to reduce the overall number of chromatography steps in their downstream processes. It can increase product yield, reduce the time required for bioprocess development, and ultimately reduce time to market and the overall cost of goods (CoG). Because of its ability to bind to the Fc region of monoclonal antibodies (mAbs), protein A has been used as the platform for affinity capture of IgG-based therapeutics for decades.

In addition to standard IgG mAbs, many antibody formats are in development: e.g., bispecifics, fusion proteins based on the crystallizable fraction (Fc) of antibodies, proteins made up of just the antigen-binding (Fab) fragments of antibodies, and antibody–drug conjugates (ADCs). Such engineered antibodies can pose a challenge to protein A affinity capture if they have altered or absent protein A binding sites. These novel formats also can be difficult to purify because some tend to aggregate, form dimers, and can come with elevated levels of high– and low–molecular-weight (HMW, LMW) species. Light-chain dimers can present alongside some antibody fragments, and overexpressed light chains can complicate their downstream processing further. In addition, some engineered antibodies are sensitive to low-pH/acidic conditions, which can limit a developer's choice of elution buffer.

New Options for Affinity Capture

Novel antibody modalities have driven development of new options for purification such as CaptureSelect technology. CaptureSelect ligands offer a unique affinity purification solution based on camelid-derived single domain (VHH) antibody fragments. The small, 14-kD affinity ligands provide a platform solution to many biopharmaceutical purification challenges and have been proven to deliver increased yields and purities for proteins of interest in many applications. VHH affinity ligands are produced by recombinant yeast culture in a process that is free of animal-origin materials. The molecules are highly stable and screened for high specificity, binding capacity, and desired elution conditions for a target therapeutic of interest. Thermo Fisher Scientific has developed a number of unique CaptureSelect affinity matrices to target different binding sites on an array of human therapeutic proteins (Figure 1).

CaptureSelect CH1-XL ligands bind to the constant heavy domain (CH1) of all human IgG subclasses and purify 100% of kappa and lambda Fabs, providing an excellent platform for Fab purification. Because of its high selectivity, the CH1-XL ligand precludes copurification of free light chains and light-chain dimers. The resin has a high dynamic binding capacity (DBC) of ~19 mg/mL for a polyclonal Fab with efficient elution at relatively mild pH. Fab purity of 98% has been achieved using CaptureSelect CH1-XL resin, with a nearly 90% yield.



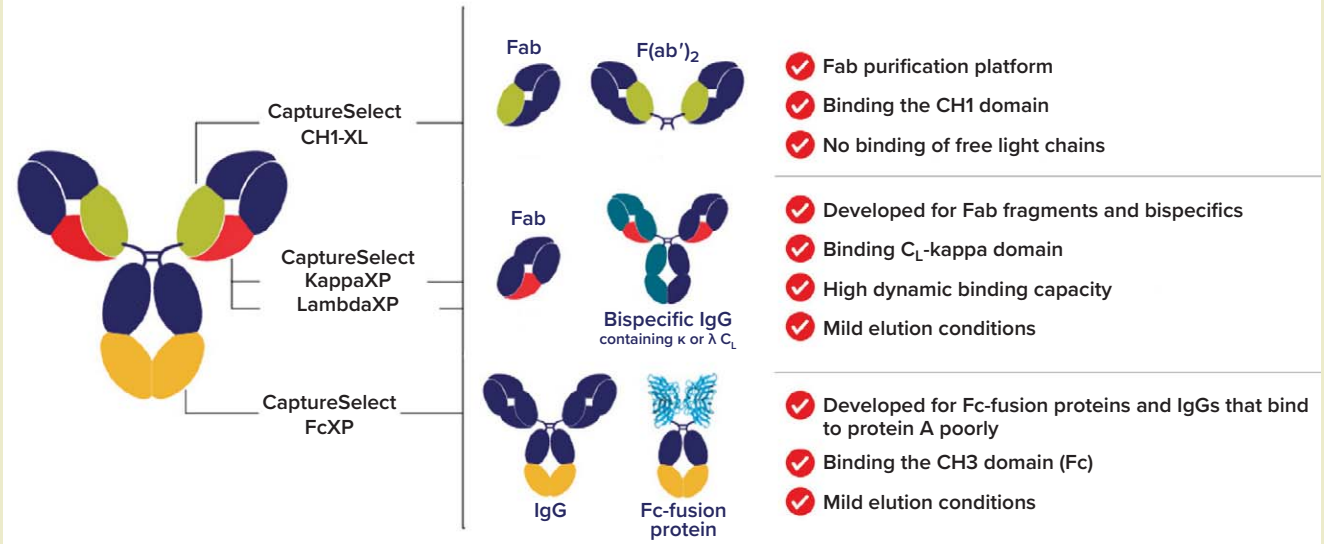
Example antibody subdomain structure

CaptureSelect KappaXP resin was designed for purification of Fab fragments and bispecific antibodies. The ligand provides 100% kappa subtype coverage for all immunoglobulins that contain a kappa light chain. This resin has a high DBC of 20–30 g/L for kappa Fabs and has a DBC of 30-45 g/L IgG. Efficient elution can be achieved at relatively mild acidity (up to pH 6).

CaptureSelect LambdaXP ligands offer 100% lambda-subtype coverage and can be used to purify IgGs that contain a lambda light chain, including bispecifics and Fab fragments. The resin has a high DBC of >35 g/L for IgGs and can be eluted at pH 3.5–4.0.

CaptureSelect FcXP ligands bind only the constant heavy domain CH3. These were developed for Fc-fusion proteins, chimeric antibodies, and

Figure 1: CaptureSelect ligands offer a range of highly specific affinity-capture options.



The first case study exemplifies rapid implementation of CaptureSelect FcXL resin into a commercial next-generation therapeutic manufacturing process. The developer wanted to REMOVE one process step to enable a better fit within a particular manufacturing facility.

Table 1: Comparing affinity-capture options for a novel-format antibody that had suboptimal binding to protein A

	(Existing) Affinity A	Affinity B	Affinity C	Nonaffinity	CaptureSelect FcXL
Load density	Medium	Low	Medium	Very High	High
HCP reduction	High	High	Very High	Low	Medium
Yield	High	High	Low	Low	High
HMW	Medium	Medium	Medium	Medium	Medium
Main peak	Medium	Medium	Medium	High	High
LMW	High	High	High	Medium	Low

molecular formats in which the protein A binding site is either removed or blocked. The resin also has a high DBC — >40 g/L with 10% breakthrough (BT) and 5-minute residence time — and elutes efficiently at pH 4.0–4.5.

Case Study in Streamlining Purification

This case study exemplifies rapid implementation of CaptureSelect FcXL resin, a predecessor of the FcXP resin mentioned above, into a commercial next-generation therapeutic manufacturing process. A purification process had been established for a novel-format antibody based on an affinity capture step followed by three polishing steps. The process had a low DBC because the antibody did not bind well to protein A, and lower-pH elution limited product stability. Other problems included elevated fragment levels and an environmentally unfriendly regeneration solution. The developer wanted to remove one process step to enable a better fit within a particular manufacturing facility.

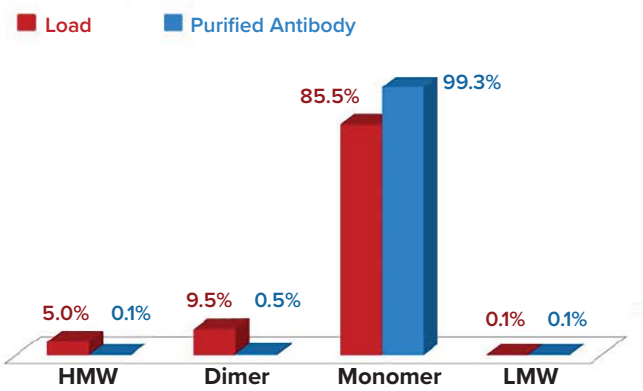
Several capture options were screened for load density, yield, and reduction of host-cell protein (HCP), HMW, and LMW impurities. CaptureSelect FcXL resin scored the best on most attributes, including the sum of LMW (Table 1). Following implementation this resin for affinity capture, the following results were achieved:

- reduction of a four-step chromatography process to three steps, removing the need for one of the polish steps
- consistent yields of ~80% in one pilot and three good manufacturing practice (GMP) runs
- increased binding capacity to improve facility fit
- an improved impurity profile
- increased pool stability due to elution at mild pH
- excellent scalability
- use of a more environmentally friendly regeneration solution.

Case Study in Improved Recovery

Using bind–elute polishing steps to remove impurities such as HMW components and dimers can be cumbersome. In this case study, a customer with a three-step purification process step sought to improve product recovery and process efficiency. The original process used protein A capture followed by anion-exchange polish in flow-through (FT) mode and a mixed-mode bind–elute to remove a high percentage of aggregates (12%). Cation exchange had been evaluated for aggregate removal but was not efficient for the

Figure 2: Results of the new process — final verification



Effective reduction of dimer and HMW aggregates in low-conductivity solutions, with high recovery

mAb-A Process	Mixed-Mode BE	HIC FT
Load density (g/L resin)	25	80
Monomer purity FT (%)	99	>99
Monomer recovery FT (%)	90	98
Host-cell proteins (ppm)	<LLoQ	<LLoQ
Residence time (minutes)	6.0	1.2

A successful mAb polish step for highly efficient aggregate removal and near-complete monomer recovery

mAb in this case. The existing process provided only 90% monomer recovery, with loading at 25 g/L and a 6-minute residence time.

The goal of our collaboration was to replace the bind–elute mixed-mode step with a FT operation to improve recovery, aggregate clearance, and efficiency. Mixed-mode chromatography was replaced with Poros Benzyl Ultra hydrophobic-interaction resin. Three attributes of such resins make them extremely well-suited for manufacturing scale: Product resolution is maintained as linear flow rate increases. High linear binding capacity is possible over a large range of flow rates. And scalability is linear and predictable.

The final process cleared aggregates to <1%, with 90% monomer recovery after 25-g/L resin loading and a 6-minute residence time (Figure 2b). The FT process was coupled directly to the upstream AEX process with no need for buffer exchange, which saved both time and money. These results demonstrate the power of HIC as an alternative strategy in mAb-aggregate polishing.

Removing HCPs with Affinity Polishing

Product- and expression-related HCPs can be difficult to remove even by multiple polishing options. HCPs that coelute with proteins of interest are particularly difficult to remove. If they are not addressed sufficiently, a product's advancement from one clinical phase to the next can be delayed. Clusterin, cathepsin D, galectin-3 binding protein, and G-protein–coupled receptor 56 are among the many HCPs that can be difficult to remove. To address such challenges, Thermo Fisher Scientific developed an approach called "affinity for polish" that applies CaptureSelect affinity ligands on POROS beads to enable fast and efficient HCP removal in FT applications. POROS CaptureSelect ClusterinClear beads were used to remove >95% of clusterin as well as >65% of other top HCPs in one customer evaluation.

The goal of our collaboration was to replace a bind–elute mixed-mode step with a flow-through operation to IMPROVE recovery, aggregate clearance, and efficiency. Mixed-mode chromatography was replaced with Poros Benzyl Ultra hydrophobic-interaction resin.

New Solutions for New Antibody Formats

Purifying the next generation of antibody therapeutics requires a diverse set of high-performance chromatography options. The tools described herein can deliver important benefits including high purity and yield in a single capture step, a reduction of the number of necessary steps in a downstream process, and seamless upscaling for biomanufacturing as products move through clinical phases toward commercialization.

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POROS resin is a pharmaceutical grade reagent for manufacturing and laboratory use only. CaptureSelect ligands and affinity matrix are for research use or further manufacturing, not for diagnostic use or direct administration in humans or animals.

A Scalable, Two-Step Purification Process for Plasmid DNA

with Simon Åberg

Plasmid DNA (pDNA) plays a critical role in biopharmaceutical manufacturing — e.g., by providing a template for mRNA synthesis, delivering genes of interest to production cell lines and viral vectors, and even serving as a basis for DNA vaccines. Although plasmid production in microbial hosts is a well-characterized process, downstream purification of the needed supercoiled (sc) forms can be time-consuming, resource-intensive, and difficult to scale. The latter concern is especially problematic considering how many applications require pDNA — sometimes large quantities of it. In a November 2023 Ask the Expert presentation, Simon Åberg (senior research engineer for viral-vector applications in the Genomic Medicine R&D division of Cytiva) described his company's efforts to improve pDNA purification. Leveraging upstream adjustments and membrane chromatography capsules, Cytiva scientists have established a scalable, two-step purification process that can be performed with single-use (SU) technology and reusable membrane capsules.

Åberg's Presentation

Process Requirements: Cytiva's legacy pDNA-manufacturing process began with fermentation of *Escherichia coli* hosts, followed by steps for cell concentration and for lysis and flocculation. After depth, tangential-flow, and normal-flow filtration (TFF, NFF) of lysed material, plasmids underwent three purification steps: size-exclusion chromatography (SEC) using Sepharose 6 Fast Flow resin, removal of open-circular (oc) plasmid forms with PlasmidSelect Xtra resin, and anion-exchange (AEX) chromatography using Source 30Q resin (all resins from Cytiva). Purified material was processed through another TFF step and then formulated for bulk fill–finish.

Although the legacy process performed robustly and fulfilled regulatory expectations, purification required considerable time, did not scale easily, and was incompatible with Cytiva's prepacked ReadyToProcess chromatography columns. Because processing involved large quantities of ammonium-sulfate buffer, the workflow also posed sustainability concerns. Cytiva scientists sought to establish a two-step purification process that yielded clinical-grade plasmids while reducing processing times, improving scalability, increasing SU compatibility, and minimizing ammonium sulfate consumption. To meet internal expectations for clinical-grade products, the updated process also needed to yield sc pDNA of >95% purity, with host cell protein (HCP), host cell DNA and RNA (hcDNA, hcRNA), and endotoxin levels well below values set forth in regulatory guidances.

Upstream Adjustments for Downstream Success: Before addressing purification improvements, Åberg underscored the importance of evaluating the entire pDNA-manufacturing process. "Optimizing upstream and clarification steps can give you advantages later in downstream," he explained. For instance, excessive antifoam concentrations can influence ratios between oc and sc plasmids, as can glycerol accumulation from ineffective feed strategies. Optimizing fermentation parameters can help to minimize generation of oc forms, reducing contaminants to be removed during subsequent purification steps.

Åberg also highlighted considerations for cell concentration and lysis. In the former case, development scientists can leverage standard centrifugation formats for laboratory-scale work; large batches could undergo microfiltration by TFF or continuous centrifugation in an SU format. Lysis steps are critical, he continued, because they must be rigorous enough to release pDNA from host cells but not so harsh that they generate considerable impurities. Performing calcium-chloride precipitation after cell lysis can be a quick, simple, and cost-effective way to separate RNA impurities before processing material downstream.

An Improved Purification Process: Cytiva scientists shortened the purification workflow from three to two chromatography steps. Lysed and filtered material is processed through a Mustang Q XT membrane adsorber capsule for capture chromatography. Then, plasmids are loaded onto Capto PlasmidSelect resin for oc pDNA removal. Compared with the legacy process, the updated workflow features higher capacity and improved pressure-flow properties. Such gains enabled elimination of the Source 30Q polishing step.

Mustang Q XT technology comes in different capsule sizes to accommodate small and large processing needs. Åberg demonstrated the adsorber's capabilities with chromatograms from pDNA purification using Mustang Q

XT5 (5 mL) and XT140 (140 mL) capsules for laboratory and production scales, respectively. The XT5 capsule was loaded with 12 mg of pDNA per membrane volume (MV) and the XT140 capsule with 9 mg/MV. Run at a flow rate of 5 MV/min, both formats had a capacity of ~16 mg/MV. They also exhibited high throughput, processing 25 mL of material per minute at laboratory scale and 700 mL/min at production scale. After an elution step with 3 M sodium chloride, the XT5 membrane process provided a step yield of 58%, and the XT140 counterpart generated a yield of 67%. Åberg noted that because pDNA binds strongly to AEX media, high yields can be difficult to achieve. Implementing multiple elution cycles can help to maximize plasmid recovery.

Åberg also presented data about Capto PlasmidSelect enrichment of sc plasmid forms. In one case, a laboratory-scale (18 mL) column loaded with 2.8 mg pDNA/mL resin was processed at a rate of 220 cm/h, then eluted using 11 column volumes (CV) of water at 120 cm/h. Column capacity was ~3 mg plasmid/mL resin with a step yield of 77%. The production-scale process leveraged a 1-L ReadyToProcess SU column prepacked with Capto PlasmidSelect resin. A load of 0.9 mg/mL was processed at 179 cm/h, then eluted at a gradient, also with 11 CV of water, at 90 cm/h. The column had a similar binding capacity and achieved a step yield of 72%. Users can apply an isocratic solution during elution, although doing so necessitates optimization of buffer conductivity.

Purification based on Mustang Q XT membrane adsorbers and Capto PlasmidSelect resin raises several technical and operational advantages. Compared with the legacy process, the improved workflow reduces processing time by 60% and requires 90% less ammonium-sulfate solution, presenting significant sustainability gains. Meanwhile, pDNA resulting from the new process continues to meet all regulatory and internal acceptance criteria for quality attributes such as sc pDNA titer (100% at production scale), hcDNA levels (0.6 µg genomic DNA/mg pDNA), HCP levels (below the limit of quantitation, LoQ), and endotoxin levels (also below the LoQ).

Associated Analytics: In addition to presenting pDNA-product data, Åberg explored analytical methods for testing in-process material. He described using agarose-gel electrophoresis (AGE) to measure hcRNA levels in samples from different downstream stages. Results demonstrated that calcium-chloride precipitation of postlysis material reduced hcRNA significantly, facilitating subsequent removal of such impurities. Åberg also showed data from chromatographic analyses of pDNA concentrations using Sepharose High Performance resin. Industry analysts often assess pDNA concentrations by microvolume spectrophotometry. But as Åberg explained, the latter method tends to overestimate plasmid titers at early process stages, when the greatest amount of hcRNA remains in sampled material. Because the Sepharose High Performance method separates such impurities from plasmids, it provides accurate titer measurement independent of sample type or stage.

Similarly, Cytiva has leveraged Capto PlasmidSelect resin for rapid screening of sc and oc plasmid concentrations. The method generates results comparable with those obtained by capillary gel electrophoresis (CGE) but can be performed in about 15 minutes. Both methods can be performed easily using an Äkta pure 25 chromatography system, facilitating data collection and saving valuable time during process development.

Questions and Answers

Why must sc pDNA isoforms be enriched during processing? Linear and oc plasmids usually are generated by "nicks" in a DNA strand. Enriching sc isoforms provides quality assurance, helping to minimize potential for mutated plasmids in final products.

Have regulators established homogeneity requirements for pDNA used to produce mRNA-based vaccines and therapeutics? This discussion is ongoing. Regulatory agencies have yet to establish clear guidelines for pDNA when it is used as a process intermediate. Perhaps such guidance will develop as mRNA-based candidates reach the biologics license application (BLA) stage. We know, however, that good manufacturing practice (GMP) in the United States requires pDNA products to have sc isoform concentrations of >95%. That requirement is unlikely to change in the near term.

Does the updated purification process eliminate steps for prechromatography buffer exchange and TFF? Cytiva highly recommends performing those steps before transferring material to chromatography. Postlysis calcium-chloride precipitation and TFF remove significant amounts of contaminants, including hcRNA, and buffer exchange is required to optimize conductivity levels for binding with Mustang Q XT membrane technology.

What column size is needed for the Capto PlasmidSelect analytical method? The method involves a 1-mL HiTrap column (from Cytiva) loaded with ~500 µL of sample.

How can the linear gradient be replaced with an isocratic elution step during purification with the Capto PlasmidSelect resin? First, the process conductivity must be optimized for a given plasmid. The key is to find conductivities that will provide a good window of separation between oc isoforms (which will flow through) and sc plasmids (which will come as eluate).

More Online

Watch the complete Ask the Expert presentation online at https://www.bioprocessintl.com/sponsored-content/a-scalable-single-use-two-step-plasmid-purification-process.

Introducing a Multitiered Classification System for Downstream Process Intensification

with Martin Lobedann

Process intensification (PI) provides a holistic framework to maximize the overall productivity of unit operations, manufacturing processes, or complete facilities. In a recent BPI Ask the Expert webinar, Martin Lobedann, PhD (process technology manager at Sartorius) described how PI reduces cost of goods (CoG), whether applied stepwise or in an end-to-end process. It can be driven by targets relating to the use or chemistry of consumables, cycling strategies, durations of unit operations, batch definitions, and levels of process integration (e.g., upstream processing batch or perfusion mode). Orchestration can interlink standalone systems to construct connected or continuous processes by optimizing tank levels and liquid flows towards periodic and steady state behavior, respectively.

Lobedann's Presentation

Lobedann defined five levels of downstream PI. **Level Zero** describes standard standalone unit (batch) operations such as single-column chromatography with a pool tank before and after the unit. **Level One** involves increased productivity and improved automation at the unit level. **Level Two** refers to a connected process of two or more unit operations, either standard or intensified, running simultaneously. The output of the first unit is immediately processed by the second in a cyclic manner. **Level Three** denotes a continuous process of multiple unit operations, totally integrated and with steady flow between them. Only small intermediate tanks are needed. Software is required to orchestrate process steps. Such an operation entails long run times and closed processing. A further increase in PI is **Level 3.1,** a complete, steady-state flow between unit operations, enabled by replacing bind-and-elute steps with flowthrough steps.

Chromatography: Most discussions about PI begin with chromatography operations, which are the most expensive and time-consuming steps in drug manufacturing. CoG can be reduced by integrating multiple unit operations, reducing a company's footprint by enabling simultaneous unit operations and requiring fewer systems overall.

In a case study of an intensified chromatography operation, the Sartorius Resolute rapid-cycling chromatography (RCC) membrane chromatography supported Level One batch purification, and the Resolute BioSMB assembly was used for Level One multicolumn chromatography. Level Two was supported by the Resolute BioSC assembly for connected downstream processing including multicolumn chromatography. Lobedann explained the differences between mass transport for chromatography media (diffusion-limited) and membranes (convective-driven). Resins can be used across multiple batches, whereas membranes can be used as single-use or single batch-use consumables.

Sartorius now offers a resin-free chromatography solution based on membrane adsorbers bearing immobilized protein A. For the bind-and-elute step, the company offers RCC to reduce membrane volume and support the lifetime capacity use, reducing CoG and increasing productivity. It requires less preparation with no packing or column requalification after storage. It supports quality control and improves stability, which lessens risks during column storage. Lobedann described how the convective membrane's small diffusion layer on the surface creates a high binding capacity. He outlined two options for RCC processing: increase membrane volume and flow rates to reduce processing time at nearly constant costs, or reduce membrane volume and flow rate to minimize costs at the same time frame.

In addition to membrane chromatography systems, Sartorius offers multicolumn chromatography systems. BioSMB systems are multicolumn units that are available in three scales. They feature high resin utilization, reducing the CoG and providing a three- to five-fold increase in productivity. Lobedann's slides detailed examples of resin loading and benefits from efficiency gains.

He concluded by describing the BioSC unit for supporting Level Two PI, which the unit connects multiple unit operations into a senior system. It can be configured for either batch or multicolumn modes, or a combination of both modes. The system supports three chromatographic steps and up to six columns.

Questions and Answers

Has PI been implemented successfully in downstream operations? PI has been successfully implemented in downstream operations due to its compatibility with perfusion.

What would be the capture column solution for Level 3.1? Because of the difficulty in capturing at this level, Lobedann recommends filtration steps to remove impurities from the product.

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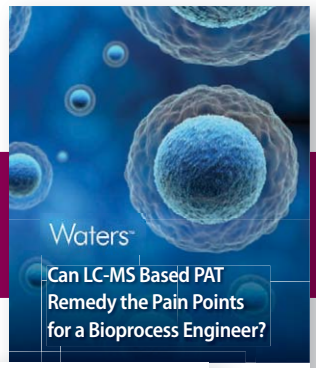

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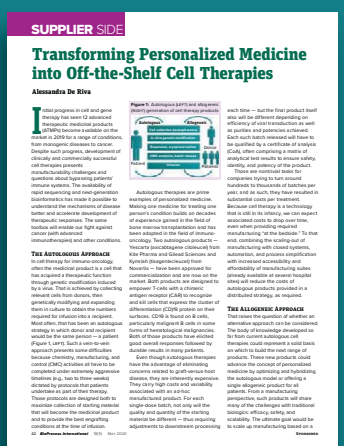
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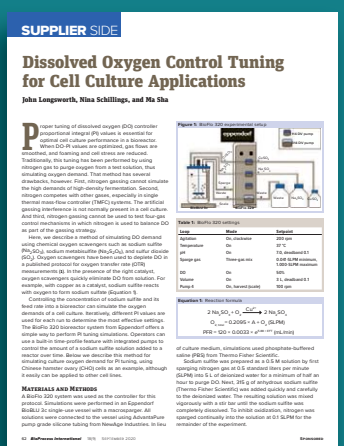
Transforming Personalized Medicine into Off-the-Shelf Cell Therapies

Alessandra De Riva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Dissolved Oxygen Control Tuning for Cell Culture Applications

John Longworth, Nina Schillings, and Ma Sha

Custom Reports

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Expansion into Late Drug Discovery and Early Development

with Derrick Katayama

The Samsung Biologics contract development organization (CDO) service has focused traditionally on cell line, process, analytical, and formulation development. Samsung Biologics has expanded its scope to support late drug discovery and increase success in early development using the S-CHOsient transient expression system and DEVELOPICK platform, as presented by Derrick Katayama (lead scientist of formulation development at Samsung Biologics). Failure rates in drug development often stem from molecular instability or selection of the wrong candidate. In this expanded program, the S-CHOsient and DEVELOPICK systems provide tools for early evaluation of molecular attributes such as cell productivity, stability risk during processing, physiochemical stability, and solubility.

Katayama's Presentation

The S-CHOsient system produces transiently expressed material and can transition from small- to large-scale manufacturing at comparable efficiency and product quality. The technology is effective across different modalities, including monoclonal antibodies (mAbs), Fc-fusion proteins, and bispecific antibodies, and provides higher productivity compared to a commercial platform with no discernible differences in product quality. The DEVELOPICK program assesses and selects the best molecule for early development activities. The tool can measure attributes such as molecular stability, solubility, and process stability. The system performs short-term analyses using small amounts of material. Automation is used to evaluate multiple candidates side by side.

Workflow: The typical DEVELOPICK workflow begins with material receipt and then moves on to assessment of primary sequences to identify hotspots in the tested molecule. The system software can compare the number of hotspots of different candidates for determination. For example, if the tested molecule is a mAb, the DEVELOPICK platform can evaluate its complementarity-determining region for hotspots and compare those with other tested mAbs. Such evaluations provide insight to minimize pathways of degradation.

After primary sequence assessment, the platform performs biophysical characterizations and basic characterization studies of tested molecules using high-throughput methods. These tests measure parameters such as colloidal and thermodynamic stability. The DEVELOPICK platform can measure the free energy of unfolding through chemical denaturation using guanidine or urea. Such tests can be performed on multiple candidates simultaneously.

Relative solubility studies also can be conducted to assess solution conditions. Typically in this stage of development, limited material availability makes it difficult to assess multiple conditions. To overcome that limitation, the DEVELOPICK platform uses a polyethylene glycol (PEG) precipitation solubility assay to monitor protein concentration. Thus, solution conditions can be compared during the same phase as molecules.

Katayama presented two case studies in selecting the most favorable candidates after testing aggregation, unfolding temperatures, thermal stress, and low-pH treatment. In one of these case studies, the chosen candidate proceeded into drug development, and the client successfully created a formulation.

Questions and Answers

What experience does Samsung Biologics have with the DEVELOPICK platform and its customers? Four drug programs have used the DEVELOPICK platform during early development since its launch in October 2022. I anticipate it being used more in the future because much attention is being given to understanding the properties of lead candidates in early development.

Would you recommend using the S-CHOsient service to conduct DEVELOPICK testing? Yes, I would recommend using the S-CHOsient system for the DEVELOPICK service. The proteins generated have good quality attributes. Early production in the S-CHOsient system also can provide early information on productivity using the S-CHOice stable cell line.

Is it efficient to spend more time conducting additional developability studies? I think that developability assessment is even more important in this fast-paced world of biopharmaceuticals. It enables selection of the correct candidates to proceed to development. Additionally, the information gained from developability assessment minimizes potential delays. Developability assessments can be performed in parallel with other activities early in the process. They do not affect the timeline, because the material used for developability assessments is also generated by the S-CHOsient system. As a result, a development program is set up for success from the start.

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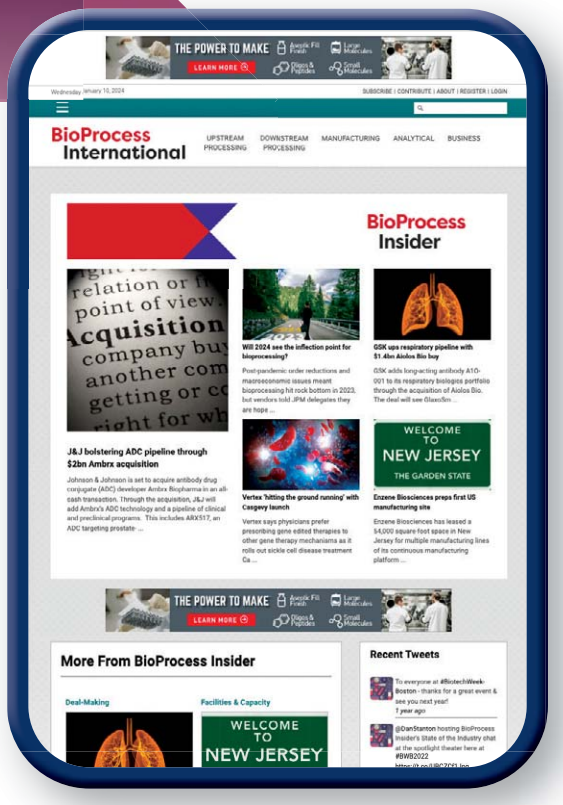

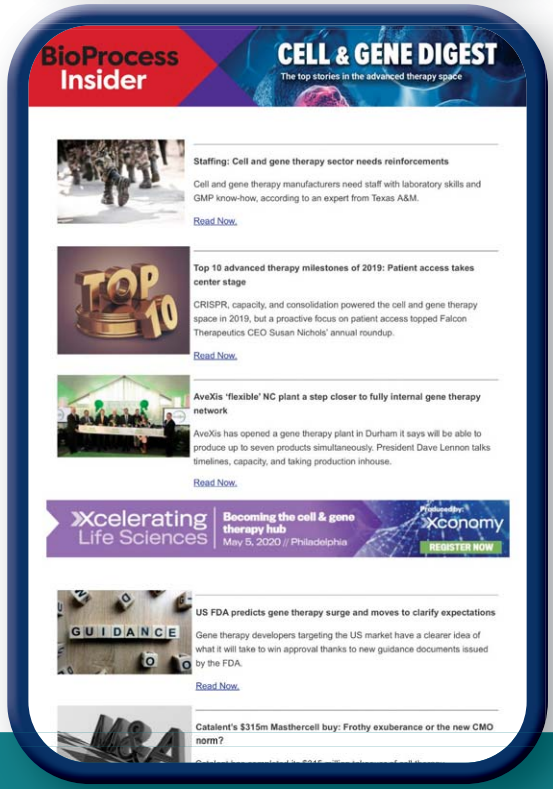

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Can Alarms Be Batch-Release Parameters?

A European Perspective

Gerardo Vece and Gaurav Walia

Alarms are integral to pharmaceutical processes and even can affect process outcomes. That raises a question: Are alarms a relevant aspect of batch release? Before our company releases a batch, we evaluate process data to ensure that everything that can affect product quality complies with regulations. Understanding whether our manufacturing process is centered and under control enables us to determine the limits within which it can shift.

We analyze alarm management by following Annex 1 of European good manufacturing practice (GMP) guidelines (**1**). It states that "process and equipment alarm events should be acknowledged and evaluated for trends. The frequency at which alarms are assessed should be based on their criticality (with critical alarms reviewed immediately)."

Certainly, staff members must acknowledge alarm events and understand the frequency with which they occur. When an alarm is triggered, it enters the evaluation of events, which workers must review before releasing documentation for regulatory compliance. Knowing such requirements, we can analyze the necessary steps to construct a compliant alarm-management system.

The first step is to develop an **implementation plan** that describes necessary tasks and timing for each activity. The plan should contain a list of relevant systems, an approach for prioritizing systems, and a Gantt chart to log activities. After enacting the plan, operators must enter denoted activities into the company's quality management system to identify responsible persons and monitor deadlines.

The next step is to **develop an assessment** that enables staff to understand the alarms of each system in scope, categorize them by criticality, identify immediate actions to resolve them, and find methods for recording both events and associated actions. This assessment should be operationalized such that learnings and identified actions can be incorporated into standard operating procedures (SOPs) for equipment use, maintenance, and cleaning.

After acknowledging alarms and evaluating their criticality, **trends must be recorded** based on historical data. If a statistically significant amount of data is available, then workers can extract information from systems that store alarm data and use that information to generate trends and alarm limits. On the other hand, if a company's systems cannot store alarm data, then operators must collect and monitor alarms manually. A database should be built for each set of equipment to help identify trends once the amount of data collected is sufficient for statistical

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processing. Finally, staff should update the revision flow in a batch's record to add verification of alarms against the predetermined limit. By completing the steps outlined above, you will have

- a complete list of your alarms
- an understanding of the criticality of each alarm
- an SOP to manage immediate actions and record activities
- trend data with averages and upper limits for the frequency with which alarms occur.

According to Annex 1, critical alarms must be evaluated immediately and according to your site's SOPs so that operators can identify possible deviation issues (**1**). Many business functions are involved in that workflow, such as those for quality assurance, production, commissioning and qualification, computer system validation, engineering, maintenance, and data integrity. With so many people involved in the process, it is essential to follow the right steps and prepare your team to provide quality oversight.

Reference

1 Annex 1: Manufacture of Sterile Medicinal Products. *The Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use.* European Commission: Brussels, Belgium, 2022; https://health.ec.europa.eu/system/files/2022-08/20220825_gmp-an1_en_0.pdf.

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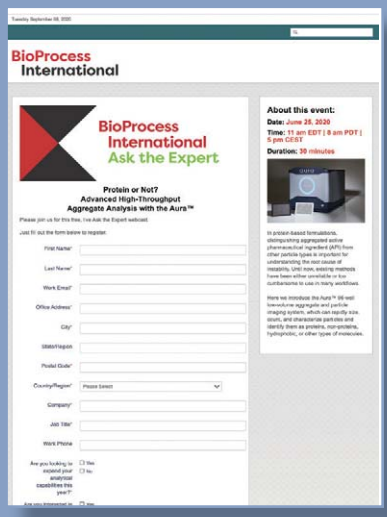

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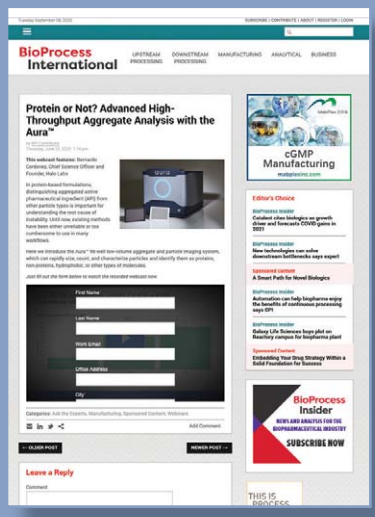

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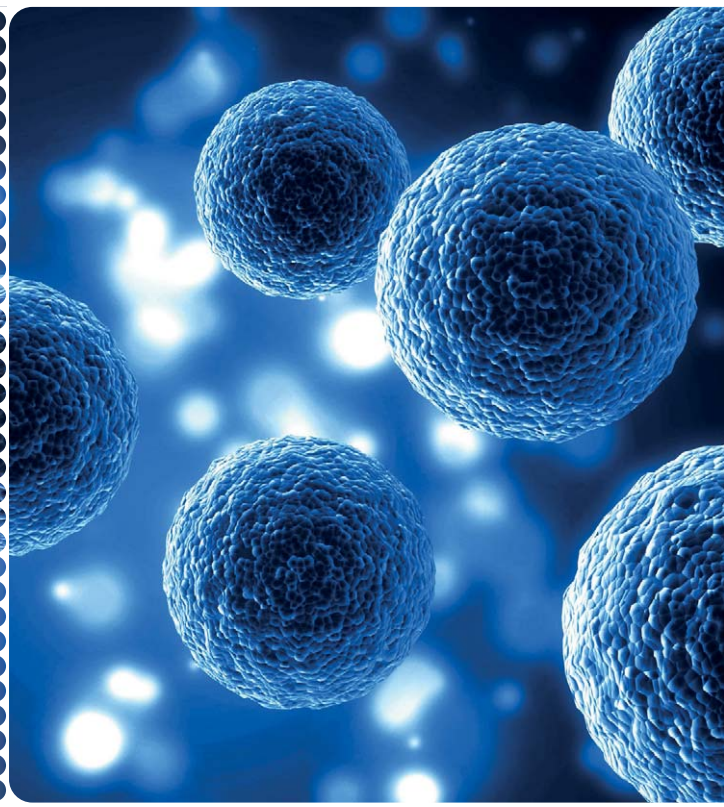

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