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**COVER STORY**

**10 Faster, Better Bioprocessing in 2020**
Achieving effective manufacturing processes and sufficient capacity remains a top priority across a diversified biologic drug pipeline.

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**FEATURES**

**EMPLOYMENT SURVEY**
Choices for Climbing the Biopharma Career Ladder
Rita Peters
With a positive employment market, some biopharma professionals explore options for career advancement.

**MANUFACTURING**
Mapping a Route for Cell and Gene Therapy Process Development
Cynthia A. Challener
While cell and gene therapies differ in many ways, some of the best practices for process development and validation are similar.

**UPSTREAM PROCESSING**
Single-Use Bioreactors: To Scale Up or Scale Out?
Feliza Mirasol
Industry experts debate the pros and cons of “going bigger” than the 2000-L industry norm in a single vessel.

**DOWNSTREAM PROCESSING**
Lyophilization Presents Complex Challenges
Feliza Mirasol
Temperature-sensitive biologics are lyophilized to preserve therapeutic viability, but the process presents complexities and challenges that are as yet not fully understood.

**PEER-REVIEW RESEARCH**
Therapeutic Potential of Green, Synthesized Gold Nanoparticles
Satabdi Rautray and A. Usha Rajananthini
This study aims to use plant-leaf extract for the green synthesis of AuNPs and to evaluate their antibacterial and antioxidant activity.

**QUALITY/REGULATIONS**
Getting to the Root of Quality Problems
Agnes Shanley
Focusing on symptoms instead of root causes locks teams into a corrective, rather than preventive, mindset.

**COLUMNS AND DEPARTMENTS**

**FROM THE EDITOR**
The promise of new therapies is tempered by the need for affordability, safety, and ethics.
Rita Peters

**REGULATORY BEAT**
Pressures on FDA will affect industry’s success in bringing new therapies to market.
Jill Wechsler

**AD INDEX**
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The promise of new therapies is tempered by the need for affordability, safety, and ethics.

Can Policy Keep Pace with Science and Discoveries?

Researcers continue to report exciting new discoveries in science and medicine that have the potential to improve life and address challenges facing society. The intersection of scientific opportunity and business profitability can, however, lead to ethical conflicts.

Emerging scientific methods such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) genome editing technology, which can be used to modify genes in living cells and organisms, offer the promise of correcting genetic mutations to treat genetic causes of disease. Researchers and ethicists generally agree that gene editing methods are suitable for research applications, but not for reproductive purposes.

Concerns about the misuse of gene-editing processes were realized when a scientist in China announced in November 2018 that he used CRISPR techniques to edit the genes in human embryos of twin girls with the intention of protecting them against the human immunodeficiency virus. This controversial work was denounced by scientists worldwide; in December 2019, a court in China sentenced the scientist to three years in prison for working outside the boundaries of scientific and medical ethics.

This experiment illustrates the ethical, safety, and legal issues associated with promising new scientific discoveries, and many unanswered questions associated with their long-term use. FDA has pursued enforcement action against dozens of unapproved therapies and treatments based on emerging sciences. In announcing a permanent injunction against a Florida-based facility for selling adulterated and misbranded cellular products, FDA noted many offenders selling unproved stem-cell products are taking advantage of vulnerable patients “by leveraging the widespread belief in the eventual promise of these products, flouting the statutes and our regulations” (1). FDA and industry experts also recognize the challenges with developing emerging therapies. FDA also noted that while stem cell products have potential to improve human health, “… that potential will never be fully realized if careful scientific work and thoughtful clinical investigation supporting the safety and efficacy of these products are not conducted.”

In the cover story in this issue, experts from bioprocessing equipment manufacturers and contract development and manufacturing organizations emphasized that standardized commercial manufacturing platforms and processes are needed to ensure that these therapies can be delivered in a safe, cost-effective manner.

Emerging therapies are expected to contribute to the growth in global sales of prescription drugs from an estimated $839 billion in 2019 to $1.18 trillion in 2024, a compound annual growth rate of 6.9%, according to Evaluate Pharma. For the top 100 products by sales, Evaluate Pharma analysts expect biotech product sales to overtake conventional product sales for the first time. In addition, biotech products are forecast to represent 32% of prescription drug sales by 2024, up from 28% in 2018 (2).

Emerging therapies represent a growing share of the prescription drug market; however, policy decisions about drug affordability lag rapid scientific developments. “The advances in cutting edge science are, for now, outpacing the traditional pricing and reimbursement systems the industry has been built on,” Evaluate Pharma reported. “This disconnect is leaving both patients and payers wondering how accessible these life-altering products will be.”

References

Coming Soon from USP Biologics:

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About USP monoclonal antibody reference standards (mAb RS):
- Recombinant humanized IgG1 expressed in Chinese hamster ovary cell culture
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hile biopharma companies can anticipate continued success in discovering new gene and cellular therapies and in devising innovative treatments for multiple serious conditions, a range of issues are poised to shape R&D and regulatory policies in early 2020. Politicians on all sides will continue to hammer drug prices, prompting industry to emphasize the importance of maintaining strong investment in R&D. FDA will remain embroiled in addressing a number of high-profile public health issues, as well as drug shortages and complex drug development initiatives.

With national elections looming in November 2020, and a host of Democrats seeking to regain the White House, health policy and pharmaceutical costs will remain leading issues for all candidates. The future of the Obama Administration’s Affordable Care Act hangs in the balance, with the debate poised to shape federal and state drug coverage and reimbursement as part of initiatives to lower outlays for medicines. Industry will challenge legislation authorizing drug importing or international reference pricing as a threat to continued innovation, but the full ramifications of election-year politicking remain to be seen.

Important challenges await FDA’s new commissioner, Stephen Hahn, most notably ongoing efforts to address the nation’s devastating opioid epidemic and to halt teen vaping of e-cigarettes, which has been linked to dozens of deaths. There’s pressure on the agency to clarify its rules for cannabidiol products, particularly dietary supplements promoting health benefits, and to address concerns about the rise in antibiotic resistance and lack of research on new treatments to combat infectious diseases. Delays in Congress approving the federal budget for the 2019 fiscal year will continue to create difficulties for the agency in assuring support for its many crucial food and drug programs.

In response to the public outcry over drug prices, FDA will continue to streamline and accelerate the approval of more generic drugs and biosimilars as alternatives to expensive brand therapies. However, most of the biosimilars approved so far have yet to reach the market due to complex patent issues as well as efforts by innovators to discourage acceptance of follow-on therapies by physicians and health plans. FDA will continue to oppose tactics to delay access to comparators needed to test generics and biosimilars, and some policy makers look to prevent brands from offering financial incentives to health plans and pharmacy benefit managers (PBMs) to favor their products. But the issues are complex and will present challenges in bringing biosimilars and generics to patients.

INNOVATION AND QUALITY

FDA has worked to establish standards to support the development of innovative, life-saving cellular and gene therapies, as seen in the approval of several breakthrough products and industry programs to develop dozens more. Patient advocates will continue to provide important voices for shaping risk-benefit equations for promising therapies, while manufacturers will invest more in modern operations able to produce quality cutting-edge products more reliably and efficiently. All parties will be watching closely to detect any safety problems or signs of limited effectiveness that could raise

Jill Wechsler is BioPharm International’s Washington editor, jillwechsler7@gmail.com.
In 2019, the 48 new drugs approved by FDA’s Center for Drug Evaluation and Research fell short of the record-breaking 59 approvals in 2018; the number of biologic-based drug approvals also was down slightly (1). Despite the drop, the number of approvals was significant, and many first-in-class therapies were introduced.

FDA’s Center for Biologics Evaluation and Research had several notable approvals in 2019. Zolgensma (onasemnogene abeparvovec-xioi), from Novartis, was the first gene therapy approved to treat children less than two years of age with spinal muscular atrophy. In addition, three vaccines were approved in 2019: Dengvaxia (Sanofi Pasteur) to prevent dengue disease in children, Jynneos (Bavarian Nordic A/S) to prevent smallpox and monkeypox disease in adults, and Vaxelis (MCM Vaccine Company) to prevent diphtheria, tetanus, pertussis, poliomyelitis, hepatitis B, and invasive disease due to Haemophilus influenza type b (2).

**References**


—The editors of BioPharm International

concerns about the long-term value of a new treatment. The importance of reliable systems for producing safe and high-quality pharmaceuticals will remain in the spotlight as part of efforts to ensure access to needed medicines. Congressional leaders are concerned about continued shortages in a number of critical treatments, as well as increased reliance on drug ingredients imported from China, India, and other countries. FDA’s Office of Regulatory Affairs (ORA) is looking to implement more fully its pharmaceutical inspectorate process, which features highly trained professionals using templates to evaluate how well production systems and practices can ensure the regular production of quality products, and to support industry efforts to modernize operations and address problems expeditiously.

More guidance from FDA should help improve testing and production of needed treatments, particularly new cellular and gene therapies that raise tricky quality control challenges. FDA will continue to support global harmonization of standards for drug production and for preclinical and clinical testing, as seen in a range of agreements with other regulatory authorities. These involve sharing and accepting reports on plant inspections, on certain testing programs, and more recently on information in market applications to achieve simultaneous drug approval decisions by multiple authorities. A main theme for the coming year will be to reduce extraneous requirements and promote more streamlined, risk-based approaches to drug development.

initiatives authorized by the 21st Century Cures Act five years ago. This time, the legislators indicate an interest in promoting wider utilization of digital health technologies to improve access to new products and health services; in modernizing coverage policies for innovative, life-saving drugs; and in utilizing real-world evidence more broadly in drug development.

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In 2020, US biopharma companies face impacts from familiar business and policy issues—access to healthcare, patent protection, the cost of prescription drugs, the biosimilars debate, and the opioid addiction crisis—as the presidential and congressional election rhetoric heats up. In Europe, the United Kingdom’s departure from the European Union is certain; however, the terms of the departure are yet to be determined. Amid these political distractions, researchers and process scientists in the biopharma industry must focus on the familiar challenge of improving current processes and increasing productivity, as well as a new hurdle related to bringing emerging therapies to market.

A more diversified biologic drug pipeline—including bispecific monoclonal antibodies (mAbs), cell therapies, gene therapies, and nucleic acids—as well as gene editing techniques and new delivery methods hold promise for patients. The tedious work to establish manufacturing processes and controls could slow progress in bringing some of these therapies to market.

Cell therapies and gene therapies continue to generate strong research and investment interest, although investments have dropped off from 2018 levels. The Alliance for Regenerative Medicine (ARM) reports companies developing cell therapies, gene therapies, and other regenerative medicines raised $7.4 billion through initial and follow-on public offerings, upfront payments from corporate partnerships, venture capital, and private placements in the first three quarters of 2019 compared with $12.5 billion in all of 2018 (1).

More than 1000 clinical trials for cell and gene therapies were in progress at the end of Q3 2019 including more than 570 Phase II and more than 75 Phase III trials, according to the ARM report. With a significant number of therapies in the late-stage pipeline, biopharma companies, contract manufacturers, and equipment and materials suppliers must invest in innovative development, manufacturing, and supply chain procedures and practices to deliver these therapies to patients.

“Monoclonal antibodies have used the same manufacturing template for the past 30 years. Novel modalities currently don’t have that same foundation,” says Andrew Bulpin, head of process solutions, MilliporeSigma. “The development of reproducible, industrialized manufacturing templates is desperately needed to provide successful and cost-effective cell and gene therapies. With that comes the need for quality and regulatory compliance standards.”

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To bridge gaps in cell and gene therapy supply—while delivering cost-effective products—continuous improvement in both upstream and downstream processing is paramount to achieve higher titers and product recoveries, says Tania Pereira Chilima, product manager, Univercells. Incorporating processes into innovative, scalable, low-footprint, low-CAPEX systems can help reduce costs, she says.

Managing both the manufacturing process and a complex supply chain is vital. “Right now, cost-effective manufacturing is not really the primary challenge for cell and gene therapies, although costs can always be improved,” Clive Glover, director of strategy, cell and gene therapy, Pall Biotech. “What is more important is ensuring that the manufacturing process is robust and delivers a consistent product. We have seen manufacturing challenges associated with several late-stage and commercialized cell and gene therapies, which in some cases have delayed or halted their approval.”

“On the manufacturing side, cost effectiveness will be driven by increased automation, dependence on high-dynamic range cell production, and closing systems to avoid overheads of classified environments,” says Phil Vanek, general manager, cell and gene therapy strategy, GE Healthcare Life Sciences. “For logistics, better integrated operational and supply chain data management, purpose-built manufacturing execution systems, and reducing dependence on liquid nitrogen in storage and shipping will help alleviate risk and costs.”

FASTER CONVENTIONAL BIO THERAPY PROCESSING
While cell and gene therapies have generated excitement, conventional biotherapeutics represent the majority of the biologics market. A few trends, however, are changing the processing strategies of some manufacturers of these products.

“There is still a lot of growth around the globe in conventional biotherapies. Cost improvements are driving the need for efficiencies in smaller scale as well as manufacture in country for country,” says Michelle Stafford, global marketing leader, BioProcess Solutions, GE Healthcare Life Sciences.

Interest in emerging therapies has been sparked by their clinical performance, says Pereira Chilima. “However, I believe that there is, and will always be, sizable investments and capacity allocation for the manufacturing of traditional/conventional biotherapeutics as these are and will continue to be standard of care for a multitude of indications,” she says. “For companies trying to diversify their product portfolio and produce both conventional and emerging biotherapeutics, investing in flexible and modular manufacturing technologies will help them cater for different product classes and efficiently respond to market fluctuations.”

Bulpin notes that more niche therapies coming to market require smaller quantities of individual biotherapeutics. “This evolution will drive continued adoption of single-use, which will enable multi-drug manufacturing facilities,” he says. “In turn, the big thrust in manufacturing will be moving towards intensified, connected, and continuous bioprocessing, as well as Biopharma 4.0.”

To resolve a huge shortage of viral-vector manufacturing space, specialized viral-vector contract manufacturers are rapidly building more capacity, and therapy developers are investing in their own manufacturing capabilities, say Glover. “These investments in the viral-vector manufacturing space have not shown impact [on] the investment in conventional therapeutics. In fact, several new sites are still under commission or being introduced for more conventional biologics at companies like Lonza and Servier,” he says.

CAPACITY VS. DEMAND
The clinical development of promising new cell and gene therapies is driving demand for capacity, says Vanek. “Capacity shortages for both [adenovirus] AAV and lentivirus are being driven by the need for [current good manufacturing practice] cGMP plasmids for vector manufacturing, plus dramatic inefficiency in manufacturing yields of viral particles. As new facilities come online for cGMP-grade vector manufacturing and new manufacturing methods are developed for improving both upstream productivity and downstream recovery, these shortages will be eased,” he says.

“There is plenty of capacity for traditional biologics manufacturing. The demand and rapid growth of novel modalities has left capacity lagging,” says Bulpin. “The standardized manufacturing templates I mentioned will bring predictable yields to inform a capacity plan. Having standardized outcomes are a key element to better capacity planning.”

Pereira Chilima cites a combination of technology innovation and process optimization as the key to addressing capacity constraints. “Process optimization in scalable advanced technologies will enable manufacturers to exponentially increase their throughputs within the same physical perimeter,” she says.

Rene Gantier, director, process R&D, Pall Biotech concurs. “This is what is driving a lot of investment and interest in the industry now by looking at ways to create a smaller footprint with an increased output to optimize efficiency, time, and money in the process,” he says. “We also see multi-product facilities enabled by single-use technologies that have flexible manufacturing approaches (e.g., manufacturing modules, ballroom manufacturing concept in controlled, not classified space).”

“To respond to market needs for capacity, speed to manufacturing, modular platforms, and workforce training are at the forefront of any business strategy,” says Stafford. “Speed to manufacturing can include in-sourcing/out-sourcing analysis, which will take into account the long-term business plan, [intellectual property] IP protection, capital expenditures, time to market,
and the technical capabilities of manufacturing or [contract development and manufacturing organization] CDMO personnel. The result of this analysis will determine if capacity is outsourced to a CDMO or organic expansion and training is warranted.

“For mammalian manufacturing, there have been significant expansions in CMO/CDMO capacity over the last year with many companies announcing new facilities,” explains Charles Christy, head of commercial solutions, Ibex Dedicate, Lonza Pharma & Biotech. “Single-use technology is facilitating rapid addition of capacity and process intensification is also playing a role in optimizing existing assets.”

THE TECHNOLOGY FACTOR
The integration of both proven and new technologies—including continuous processing, automation, virtual reality (VR), and data management—are vital to meeting and advancing bioprocess capacity demands.

Automating bioprocesses is a crucial factor to realizing the benefits seen for other industries such as reduced time to market and time to process, process and product safety through reduced operator errors, improved consistency and reproducibility, says Gantier. “The systematic use of quality-by-design [QbD] principles, where all process variables are characterized in detail, enables building accurate and reliable process models. Also, advancing sensing technologies and analytics from monitoring to predictive, then prescriptive and finally cognitive helps to enable highly automated bioprocesses.”

“Data availability and mine-ability will provide us with better insights for process optimization and manufacturing efficiency improvements,” says Vanek. “While still early, the data we're collecting as an industry can be curated to connect events with outcomes, and this, in turn, can lead to new insights and ultimately predictability of processes. Connecting sensors, biological data, and manufacturing performance in real-time will also enable improved automation and better decision making.”

“Big data and artificial intelligence will move us to descriptive vs. prescriptive methods of manufacturing control,” Bulpin notes. “A descriptive method moves away from a traditional biologic manufacturing standard operating procedure and tunes into the needs of the living organisms used to produce a biologic. Big data will develop the ‘golden batch,’ delivering consistent and optimized outputs in each run.”

“Although ‘Industry 4.0’ is not a totally new concept, it has taken more time to integrate new technologies into the complex and risk-averse bioprocessing world,” notes Christy. “There are many areas where advances in digital technology can and are making a difference, ranging from onboarding new operators with VR, reducing time for tech transfers and product release using more advanced data analytics, and modeling bioprocesses to optimize QbD.”

WHAT’S NEXT FOR MANUFACTURING PROCESSES?
Pereira Chilima predicts that the industry will continue to focus on achieving higher production titers and move towards intensified, automated, and continuous processes to produce ‘more for less.’ This approach will enable manufacturers to dramatically reduce the footprint of operations, achieve higher productivities per production line, and install more production lines within the same footprint. “This will result in higher annual throughputs, lower CAPEX, and consequently lower cost of goods sold (COGS),” she says.

“Although process intensification and continuous manufacturing have been a discussion point for years, joining the different elements together or focusing on hybrid processes will move us one step closer to fully joined up solutions,” predicts Christy. “Real-time release is already a reality in small-molecule manufacturing and something that will considerably speed up biologics manufacturing.”

“The application of continuous manufacturing to monoclonal antibody production has been well studied. However, its ability to impact novel therapies such as viral vectors has not been well characterized,” says Gantier. “There are, of course, complications to applying continuous manufacturing to viral vectors, not least of which is the fact that the virus kills the cell that produces it. Nevertheless, there is the possibility for continuous manufacturing to drive unit operation intensification bringing greater process robustness and reproducibility.”

For cell and gene therapies, Vanek expects a better understanding of biological systems and how they are impacted during manufacturing, improved process analytics to monitor that biology, and digital integration to collect data and make process decisions based on real-time biological information.

Industrializing cell and gene therapies will continue, moving along the same commercialization and optimization curve as recombinant proteins and monoclonal antibodies, says Christy, “but hopefully faster given that the industry can apply some of the experience learned in fermentation and cell culture.”

“We also expect a resurgence in microbial manufacturing as some of the newer complex proteins in early phase not requiring mammalian glycosylation or the linker elements for some biocogjugates become more important. Improvements to expression systems, titers, and process intensification can further reduce COGS and production times considerably in this platform, providing distinct advantages for classes of molecules such as novel vaccines, protein–linker biocogjugates, and even bio-similars,” Christy concludes.

REFERENCE
1. Alliance for Regenerative Medicine, Quarterly Regenerative Medicine Sector Report (Q3 2019).
Choices for Climbing the Biopharma Career Ladder

With a positive employment market, some biopharma professionals explore options for career advancement.

RITA PETERS

An employee’s view of the job market is often colored by recent work experiences—whether positive or negative—and their most recent salary increase. Professionals working in biopharma drug development and manufacturing are concerned about the size of their paychecks, but other factors—including challenging work, job security, and company stability—may be more important when making career change decisions.

Insight provided by bio/pharma professionals from around the world responding to BioPharm International’s annual employment survey (1) indicates strong confidence in the biopharma industry (66% expect business improvement in 2020) and some confidence in prospects for their employer (52% expect business improvement in 2020). More than half of the respondents, however, expressed interest in seeking better opportunities beyond their current position. (See the infographics on pages 16–17 for an overview of survey results.)

Job insecurity, company restructuring, a lack of training, an unsatisfactory work/life balance, and uncertainty about the company’s performance or success were the top reasons for job dissatisfaction.

Similar to previous surveys (2-3), more than half of all respondents said they would like to leave their jobs, given the opportunity; however, 58% said they do not expect to leave in the coming year. A significant segment, 19%, said they would like to change careers and leave the bio/pharma industry.

Employees working in the biologic drug segment reported more satisfaction with their current positions. Only 17.3% of biologics respondents agreed strongly that they would like to leave their job, given the opportunity, compared with 25% for those working in the small-molecule segment.

PRIME TIME FOR A JOB CHANGE?

Survey results indicate bio/pharma professionals are on the move. More than 44% of 2019 respondents—compared with one-third in 2018—said they stayed with the same employer, on average, for five or fewer years. Respondents working in large-molecule drug development and manufacturing stayed with the

Article contin. on page 18
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Employment Survey

Does your current salary reflect a change over last year’s salary?

<table>
<thead>
<tr>
<th>Change</th>
<th>2019</th>
<th>2018</th>
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<tbody>
<tr>
<td>Increase</td>
<td>55.4%</td>
<td>54.1%</td>
</tr>
<tr>
<td>Decrease</td>
<td>11.6%</td>
<td>12.3%</td>
</tr>
<tr>
<td>No change</td>
<td>32.2%</td>
<td>33.6%</td>
</tr>
</tbody>
</table>

How secure do you feel in your job compared with last year?

<table>
<thead>
<tr>
<th>Security</th>
<th>2019</th>
<th>2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>More secure now</td>
<td>33.4%</td>
<td>44.7%</td>
</tr>
<tr>
<td>Less secure now</td>
<td>30.6%</td>
<td>30.6%</td>
</tr>
<tr>
<td>No change</td>
<td>36.0%</td>
<td>24.7%</td>
</tr>
</tbody>
</table>

Please rate your satisfaction with your current salary.

I am paid below market value, considering my level of expertise and responsibility. 17.6% 20.5%

I am paid within market value for my job function, but at the low end of the range, considering my level of expertise and responsibility. 40.9% 38.2%

I am paid fairly for my level of expertise and responsibility. 38.9% 39.2%

I am paid excessively for my level of expertise and responsibility. 2.6% 2.1%
Bio/pharma workers contemplate job and career changes.

- I would like to leave my job, given the opportunity. (24.3% in 2019, 21.2% in 2018)
- I do not expect to leave my job in the coming year. (24.9% in 2019, 29.6% in 2018)
- I would like to change careers and leave the bio/pharma industry. (36.5% in 2019, 21.7% in 2018)

If it were necessary for you to change jobs this year, how would you assess the job market?

2019
- It would be straightforward to find a job comparable to the one I have now. (24.4%)
- It would take a while, but I would be able to find a job comparable to the one I have now. (40.6%)
- It would be straightforward to find a job, but it probably wouldn’t be as good as the one I have now. (17.8%)
- I would have to search hard and be prepared to take what I could get. (9.6%)

2018
- Competition for open positions is strong. (35.2%)
- Competition for open positions is moderate. (36.8%)
- Employers compete for qualified candidates. (28.0%)

In your career, how long, on average, have you stayed with the same employer?

- Less than 2 years (34.5%)
- 3 to 5 years (14.7%)
- 6 to 10 years (11.2%)
- 11 to 20 years (9.6%)
- More than 20 years (14.7%)

Which statement best describes the job market for scientific or technical positions in bio/pharmaceutical development and manufacturing in your geographic area?

- Strongly Agree (28.0%)
- Somewhat Agree (36.8%)
- Somewhat Disagree (35.2%)
- Strongly Disagree (1.6%)
same employer longer than those in the small-molecule market segment.

More than one-quarter of the respondents said they voluntarily changed jobs in the past two years. The reasons cited—with multiple choices allowed—were to pursue a better career opportunity (72.7%), find more challenging work (40%), or to seek a better work-life balance (32.7%). Those working in the biologic drug segment valued company stability more than those in the small-molecule segment, who said job security was more important.

Salary was the fifth most-cited single reason for job change, trailing work-life balance, professional advancement, intellectual challenge, and job security. Nearly two-thirds of the respondents were confident they could find a job similar to their current position, should they choose—or were forced—to find new employment.

In the past two years, nearly one-quarter of the respondents said their company experienced a merger or acquisition, up from 18.1% in the previous survey; an additional one-quarter of the respondents reported that their companies had been through a downsizing or restructuring. Nearly 20% of the respondents said they left the company due to such an acquisition, downsizing, or restructuring.

Respondents suggest there is a positive market for job seekers; 36.8% of respondents said there are few qualified candidates for open scientific/technical positions (almost 40% for biologics positions), compared with 30.3% in 2018. A smaller percentage (28% in 2019 vs. 34.2% in 2018) said there were more qualified candidates than open positions. As in previous surveys, respondents expressed somewhat negative opinions about the knowledge and skill sets of new hires; 73% said the new hires were adequately trained but not exceptional; 18% said they were poorly trained.

In 2019, more respondents said they felt more secure in their position compared with the previous year (30% in 2019; 25% in 2018; 19.1% in 2017). Another 30% said they felt less secure, similar to the response rate of previous years. Interestingly, those working in the biologic drug sector felt more secure in their jobs than those in the small-molecule sector. In addition, only 22.6% said they felt less secure in their jobs than the previous year, compared with 34.5% in the small-molecule sector.

THE COMPENSATION FACTOR

Salary ranked sixth on a list of 12 factors contributing to job satisfaction, up from ninth place in 2018. Compensation trailed factors such as challenging projects, intellectual stimulation, company’s potential for success, a good work-life balance, and supportive management as the “main reason I come to work.”

Satisfaction with compensation has trended downward during the past few years. More than 39% said they were paid fairly or excessively in 2019, compared with 41% in 2018 and 46% in 2017. In 2019, more than 40% of those surveyed said their pay was at the low end of the salary range for their expertise and responsibility; 17.6% said they were paid below market value.

The number of people reporting salary increases was stagnant from 2018 (54.1%) to 2019 (55.4%), following a drop from nearly 63% in 2017. The number of people reporting a decrease in salary ticked up from nearly 9% in 2018 to 11.4% in 2019.

More than one-quarter of respondents said they used their full allotment of vacation, personal, and sick time while 37% said they used less than half of the available time off.

RESPONDENT PROFILE

More than 220 bio/pharma professionals from around the world responded to the survey, which was fielded in November and December 2019. Respondents primarily were from innovator bio/pharmaceutical companies (31.3%), generic-drug manufacturing companies (13.3%), and contract research and manufacturing or consulting organizations (17.6%).

The respondents were involved in developing or manufacturing a range of drug types—with some listing multiple types—including small-molecule drugs (58.9%), large-molecule drugs (45.1%), vaccines (19.8%), cell therapies (17.8%), gene therapies (17.8%), and nutraceuticals (17.3%).

Respondents work at small and large companies and are responsible for R&D, process development, technology transfer, validation, quality control/assurance, formulation, manufacturing, and other functions. Nearly 40% of the respondents held a doctorate or higher degree; more than one-third held at least a Master’s degree.

Compared with previous years, the respondents had slightly less experience working in the bio/pharma industry; 26.9% had fewer than 10 years of experience, 25.6% had 10–20 years, 36.9% had 20–35 years of experience, and 12.1% have worked in the industry for more than 35 years.

Respondents working in the small-molecule drug arena worked more hours compared with peers in the biologic-drug segment. More than 37% reported working more hours than the previous two years, compared with 30.7% for the biologics workers.

REFERENCES

1. BioPharm International, 2019
   Pharmaceutical Technology/BioPharm International Employment Survey, December 2019
2. BioPharm International, 2018
   BioPharm International Employment Survey, December 2018
3. Pharmaceutical Technology, 2018
Single-Use Bioreactors: To Scale Up or Scale Out?

Industry experts debate the pros and cons of “going bigger” than the 2000-L industry norm in a single vessel.

FELIZA MIRASOL

As demand for biologics continues to increase, manufacturers must process higher volumes upstream, and more are turning to single-use bioreactors. Demand for single-use reactors has been growing by 13.5% per year, and is expected to continue at this rate through 2026, when it will exceed $1.5 billion, according to a report by Transparency Market Research (1). Mammalian and bacterial cell culture accounts for more than 85% of demand, according to the analysts.

Technology vendors have responded to the need for higher downstream capacity with larger-volume single-use bioreactors. Where 2000 L was once the upper bound, some bioreactors are now available in any volumes up to 6000 L, with 4000 L currently in operation (2,3). Experts question whether single-use bioreactors can operate efficiently above 2000 L, however (4,5).

One fundamental question focuses on the structural soundness of the reactors, and whether leaking or other issues can be exacerbated at higher volumes. According to Transparency Market Research’s report (1), the plastic extrusion process used to manufacture single-use bioreactors can leave them vulnerable to leaking. Vendors have been optimizing equipment by stabilizing materials in order to reduce the risk of leaks.

THE DEBATE OVER GOING BIGGER

When considering the capacity constraints for any single-use bioreactor, a number of important considerations must be kept in mind, says Kevin D. Ott, executive director at Bio-Process Systems Alliance (BPSA), whose engineering experts also weighed in on the topic. “In essence, economies of scale must be weighed, system integration planned for, and qualified engineering firms engaged in order to plan for the larger footprints and benefits of larger bags. This entails a user/supplier/engineering house relationship that starts early and is project-specific,” Ott says.

It is not necessary to limit capacity, says Ott and his colleagues, but, he notes, “users/adopters need to plan, and plan some more, with suppliers as their partners, for integrating these bioreactors effectively into existing manufacturing facilities.”

“Classified areas need to get bigger to handle a larger footprint, and ceiling heights must rise to accommodate larger systems. Building floor loads are also a concern, especially with retrofitting in a previously built suite,” he says. In addition, BPSA experts note that, as the size of the bioreactor increases, so will the supporting infrastructure required for gas supply, power, waste handling, and jacket temperature maintenance.
Bag size needs must also be taken into account, they say, because they will have an impact on such decisions as bag ports, tubing size, sterile connectors, and pump and filter capacity. Manufacturers must also consider the increased power that larger reactor outputs will demand.

Choice of size will also impact storage and handling. Larger single-use bioreactors may also require re-alignment of the warehouse shelving and demand that more attention be paid to material handling to ensure bag integrity. Finally, BPSA experts say, the larger bioreactors will also need to be validated/sterilized, emphasizing the importance of sterilization chamber size.

“There is no ‘one-size-fits-all’ bioreactor,” notes Parrish Galliher, chief technology officer, Upstream, at GE Healthcare Life Sciences, but 2000 L is the commonly accepted maximum commercial-scale volume, he says. “The productivity of the 2000-L scales system has found a niche in the industry as titers have increased over the last 10 years—they have been on the market for more than a decade and have also been proven commercially viable at many licensed facilities across the globe. Most companies choose to lower the risks of scaling out, investing in multiple identical 2000-L production lines versus scaling up,” he says.

“The industry has settled on 2000-L platforms as a norm in the design of the next-generation biotech facilities,” agrees Ott. “Going larger” brings with it the challenges of integration, handling, storage, and removal,” he and fellow experts at BPSA point out.

Others have a different view. “It is well known that the standard 2000-L systems currently on the market cannot achieve the mixing and mass transfer performance of comparable stainless-steel systems, with performance dropping off over a few hundred liters,” notes Brady Cole, vice-president of equipment solutions, ABEC, which launched its 4000-L and 6000-L single-use bioreactors in 2017 and 2019, respectively. Cole points out that the performance of the standard 2000-L systems is not optimal for many high-density cell culture applications.

**PROCES S INTENSIFICATION**

At this point, some observers say, the industry prefers smaller single-use bioreactors and process intensification over scaling up with larger bioreactors. “The volume requirements for commercial cell-culture bioreactors are getting smaller, not bigger,” says Melisa Carpio, technology consultant, cell culture technologies at Sartorius Stedim Biotech. “According to BioPlan’s 2019 Biomanufacturing Report, the greatest demand was for 1000-L single-use bioreactors, followed by 500 L, and then 2000 L,” she says.

When manufacturers implement larger single-use bioreactors (e.g., 4000-L models), they are usually doing so to address a specific facility need (e.g., facility fit), notes Hemanth Kaligotla, segment marketing manager, monoclonal antibodies, at Sartorius Stedim Biotech. Larger volume single-use bioreactors may also be used by contract development and manufacturing organizations that continue to manufacture legacy low-titer products for large biopharma companies, he says.

**ASSESSING POTENTIAL RISKS**

When considering the size of a single-use bioreactor, the risk of increasing overall size should be assessed, and the following considerations should be made, says Galliher:

- Ease of logistics and handling (i.e., warehouse space, ease of unpacking, inspection, loading into the tank, and disposal at the end of the run)
- Time considerations (i.e., the amount of time needed to fill or drain the bioreactor due to limitation in the size of filling and draining ports—a 2000-L bioreactor generally takes two hours to fill or drain)
- Impact on other equipment as the bioreactor scale goes up (e.g., the size and cost of the larger inoculum preparation train required to inoculate larger bioreactors with potentially more tanks and more bags needed per batch; the increased size and cost of media and buffer prep systems; and the size and cost of the larger harvest and capture step required to harvest larger volumes)
- Technical and operational costs, including the increased risk of scale-up problems, such as negative effects on cell viability, product quality, and final yield; additional costs of larger bioreactor bags with additional and/or larger impellers; and the risk and increased cost of contamination as scale increases.

Often, failure to optimize processes will dictate use of larger single-use bioreactors. “Processes that have not been optimized will generally produce lower titers and will require larger bioreactors. For these processes, a 4000-L might be required to meet market demand,” Galliher says.

Cole, meanwhile, explains that 4000-L single-use bioreactors provide much better economies of scale because batch sizes are doubled for a similar amount of floor space, and mixing and mass transfer performance (therefore titer) is comparable to stainless-steel systems of that scale. Importantly, the productivity benefits are achieved with no added risk to cell viability and product quality because the designs and scaling are based on methods proven for decades, and the bioreactors can be customized for wide process windows.

**MAINTAINING CELL VIABILITY AND PRODUCT QUALITY**

Among the challenges to deal with when selecting a single-use bioreactor larger than 2000 L are cell viability and product quality, says Galliher. “The strength and scientific and engineering expertise of the process development team will be tested more extensively as bioreactor scale increases, and cell viability and product quality are potentially at higher risk,” he comments.

It can take a long time, and be very costly to unravel and rectify cell viability and product quality problems, resulting
in delays to market, which is another reason why more companies are choosing to scale out rather than up to a larger single-use vessel, Galliher says.

In addition, as bioreactor scale goes up, clean-up, disposal, media requirements, and in-line/at-line monitoring become more expensive. “Waste disposal permits for larger volumes and solid waste may be more difficult to obtain, or may not be permitted,” he explains.

Carpio adds that high pressure (especially around port windows) and the consistency of control (e.g., of temperature) are other potential challenges to working with larger single-use bioreactors. The larger devices will also weigh more, posing installation challenges, she says, and scalability will be another factor to consider. “Any single-use bioreactor larger than 2000 L, especially a bioreactor with a different geometry from most 2000-L bioreactors, would need to show scalability with regard to cell growth, productivity, and product quality,” Carpio says. She notes that Sartorius Stedim Biotech has emphasized the need for geometries that enable single-use scalability to match that of traditional stainless-steel devices from 250 mL to 2000 L.

An advantage to scaling up to a larger capacity vessel, however, would be to reduce the need for production-stage bioreactors to meet market demand. This has often been the primary rationale behind scaling up (rather than out), because it may reduce some limited capital and space costs, according to Galliher. Another advantage is that larger scales result in lower manufacturing labor and quality control costs per gram of drug produced. “These surface benefits may be overwhelmed, however, by the total risks and cost,” he says.

Going bigger also offers several advantages with respect to cost and flexibility, says Cole. These advantages include:

- **Lower cost of goods with approximately two times the productivity per unit floor space, giving process performance, and therefore yield, similar to stainless-steel systems**
- Lower capital cost as fewer systems are needed to achieve high capacity
- Lower facility cost since less floor space is needed with fewer systems needed
- More process flexibility in that production quantities needed for a given product can be achieved with fewer batches, thereby freeing up capacity for other products.

### CAPACITY SIZE OF THE FUTURE?

Despite the risks involved, “going bigger” than the current industry norm is not only feasible, but Ott and BPSA experts encourage it. “BPSA has had such discussions during its annual summits held in Washington, DC, within our ‘factory of the future’ presentations,” he says.

“For system performance at larger scale, preparation, planning, and implementation are essential for larger(er) systems to perform to expectations, and to yield the expected economic benefit(s) to the user-adopters,” Ott adds.

“We have demonstrated 6000 L and hypothetically could go larger,” says Cole. “The limitations would be based more on practical considerations rather than engineering hurdles.”

Galliher is cautious about making the move. “The holistic risks and costs at the [larger] scale are already pushing many of the critical issues,” Galliher says, singling out mixing, mass transfer (oxygen and carbon dioxide management), and heat transfer. “Stainless-steel bioreactors are operating old legacy processes today at 20,000-L scale and, in fact, may have lower operating economies when fully utilized. Designing a single-use liner at 6000-L–10,000-L scale that keeps all the potential collateral risk and costs at bay will probably be prohibitively risky and costly,” he says.

Meanwhile, Kaligotla believes that more analysis is still needed to evaluate the technical and economic feasibility of using larger commercial scale single-use vessels. In the short term, manufacturers might implement larger capacity single-use bioreactors, but this may not be sustainable in the long term, he notes. One benefit would be in media, which are likely to developed at lower cost in the immediate future, he says.

“While it is potentially feasible to go to 4000-L, 6000-L, or even higher-volume single-use bioreactors, the feedback we have received from customers suggests that flexibility, performance, and robustness are crucial drivers,” adds Carpio.

“With the industry moving towards process intensification, the focus is on smaller operating volumes and the ability to run different, higher performance processes on the same equipment.”

So far, she says, customers tend to prefer smaller volume single-use bioreactors (e.g., 500-L or 1000-L models), or to scale out processes employing multiple 2000-L bioreactors in a “ballroom” approach, depending on product quantity needs for a particular process. “There is also skepticism for extra-large volumes with regard to scalability and user operation,” she states.

### REFERENCES

Lyophilization Presents Complex Challenges

Temperature-sensitive biologics are lyophilized to preserve therapeutic viability, but the process presents complexities and challenges that are as yet not fully understood.

FELIZA MIRASOL

As more biological molecules are explored as therapeutic agents to treat diseases, the use of lyophilization is increasing. Despite its challenges, lyophilization remains the best option to preserve molecular integrity and, therefore, therapeutic efficacy. However, the process is complex and challenging. Optimizing it requires a clear understanding of underlying mechanisms and issues.

At its most basic, lyophilization is a freeze-drying process by which water or liquid is removed from a product after it has been frozen and placed under a vacuum. Lyophilized product can be stored safely for much longer periods than unlyophilized product, avoiding such problems as loss of biological activity and unpredictable, undesirable immune responses. The process also minimizes damage to the material that would occur if it were to be dried only. Living cells, proteins, and other complex molecules survive well with lyophilization and can easily be rehydrated after prolonged storage periods. In the case of a biological therapeutic agent, the biologic is rehydrated and put into solution prior to administering to the patient.

LYOPHILIZATION CHALLENGES

Although costly and time consuming, turning a therapeutic agent into a dried powder formulation via lyophilization has been the industry’s most practical solution to stabilizing the biologic. Lyophilization also offers other advantages, such as providing a desired expiration limit for proteins that are naturally volatile or that easily and quickly break down, providing stability at ambient temperatures, inhibiting proteolytic enzymes from breaking down the protein, and allowing for the potential modification of the biologic formulation after manufacture and prior to administration (e.g., adding preservatives, changing pH or viscosity, or adding diluents for special applications) (1).

Despite the advantages that lyophilization offers, bio-manufacturers still struggle with the challenges of the process, which can often be attributed to the complexity...
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of biological molecules, such as therapeutic proteins, antibodies, antibody drug conjugates, and, now, cell and gene therapies. For instance, common components used in the manufacture and formulation of proteins, monoclonal antibodies, and other biologics can destabilize molecules in freeze-dried, or even frozen, formulations. These include such common components as buffers and surfactants used during the manufacturing process (2). Phosphate buffers, such as sodium phosphate, may contain one component that may precipitate during freezing, which could change the pH of the solution in which the biological molecule is carried. The shift in pH could destabilize the molecule during lyophilization, thus it is important to understand how commonly used bio-manufacturing components can act during the freeze-drying process. It is recommended that minimum buffer concentration needed to control pH, regardless of buffer type, be used to avoid instability in the biological molecule during lyophilization (2).

The nature of complex biological molecules poses other challenges to the lyophilization process. For one, biologics are sensitive to various interactions, including those that occur at the air/liquid interface, at the liquid/primary packaging interface, and even at the solution/ice interface. These interactions typically cause the protein to unfold, which can result in aggregation and, thus, loss of bioactivity. The end result would be the loss of therapeutic efficacy in the patient. Another challenge is freeze concentration; when ice forms, biologics become more concentrated and molecules come into close contact with each other. This can potentially lead to pH shifts as well as increases in ionic strength. Finally, because protein structure and bioactivity depend on the molecule’s interaction with surrounding water, removing water during lyophilization destabilizes the protein structure; thus, for a biologic, some residual moisture must be retained in the final lyophilized cake to ensure stability (3,4).

In addition to the challenges of dealing with complex, biologically active molecules, there is also a need for specialized knowledge. Because there is no “one-size-fits-all” lyophilization process, each biologic product requires an extensively studied and designed process specific to that molecule. For example, extensive studies may be needed to understand the freezing and drying behavior of components used for the formulation of the biologic, and studies should be done to determine how factors such as formulation strength and/or containers affect the freeze-drying process. Complex formulation components, such as nanoparticles, microparticles, or liposomes (5), would also require study and further knowledge of their behavior and characteristics during freeze-drying. In addition, mechanical factors such as temperature, pressure, and time setting, differ with each type of molecule, and thus these parameters should be taken into consideration when designing a lyophilization process for a specific biologic (6).

Another challenge is that lyophilization presents a high contamination risk because product is exposed for extended periods of time. It is therefore recommended that sterile lyophilization be conducted in an ISO 5 (Class 100) environment (5). Clean-in-place and steam-in-place capabilities should also be in place.

MORE DEMAND

Despite the challenges, time, and costs of lyophilizing biological molecules, the need for lyophilization is growing, driven by the increasing number of innovative biologic product candidates and biosimilars that are now being developed. Industry observers have even noticed growing demand for lyophilization in the small-molecule pharmaceutical sector, as more small-molecule drugs are being produced in an aqueous medium (6).

The biopharmaceuticals market is also seeing the incursion of the first round of biosimilars for blockbuster innovator biologics, and many of them will be lyophilized products. As innovator patents reach expiration over the next decade (4), more biosimilars are expected to reach the market. In this case, biopharmaceutical companies developing biosimilars have an added challenge of demonstrating that the lyophilized biosimilar looks and acts the same as the innovator biologic it references. Regulators will scrutinize any biosimilar formulations, which must closely match the formulation of the innovator products.

REFERENCES


Mapping a Route for Cell and Gene Therapy Process Development

While cell and gene therapies differ in many ways, some of the best practices for process development and validation are similar.

CYNTHIA A. CHALLENER

The regenerative medicine field is still quite young, and companies developing commercial processes for the production of cell and gene therapies face several hurdles. These products are highly complex and often involve production methods that, while in some ways are similar to those used in biologics and vaccine manufacturing, in many ways are quite different.

Often the level of understanding of the product and process requirements is less than desired. This results in part due to the complexity of the product, but also due to a lack of analytical tools that can provide relevant information with sufficient sensitivity. There is also a need for appropriately designed equipment and technologies. These factors make process development and validation for cell and gene therapies a challenging and highly critical task.

SCALING DIFFERENCES

Depending on the application (i.e., autologous vs. allogeneic cell therapies or gene therapy vs. cell therapy applications), the focus of scaling of the manufacturing process may be different.

Scale up and mass production of the final product starting from a small seed bank (for instance, a master cell bank or working cell bank established from a healthy donor) is the main focus of the allogeneic applications, according to Behnam Ahmadian Baghbaderani, global head of cell and gene therapies for Lonza Pharma & Biotech. In comparison, for autologous applications, scale out and parallel production of multiple manufacturing processes (each starting from a patient sample) is the focus of scaling strategies.

Looking at cell therapies, Baghbaderani notes that the main differences in scaling allogeneic applications lies in the cellular characteristics, cell culture systems, and unit operations utilized in these processes, including the downstream methods employed to produce the final product. “The cellular characteristics (i.e., anchorage-dependent versus anchorage-independent cell types) or the biological mechanism through which the final cell therapy product is...”

CYNTHIA A. CHALLENER, PhD, is a contributing editor to BioPharm International.

Contin. on page 28
An integrity-assurance strategy for single-use systems can mitigate risks and uphold regulatory compliance in biopharmaceutical manufacturing.

As adoption of single-use systems in more critical process steps of biomanufacturing increases, strategically maintaining and protecting the integrity of such systems is paramount to producing safe and efficient biologic drugs.

*BioPharm International and Pharmaceutical Technology* recently sat down with Marc Hogreve, Principal Engineer for Integrity Testing at Sartorius Stedim Biotech, to discuss the company’s integrity-assurance strategy for single-use systems.

**BioPharm/PharmTech:** Why is single-use system integrity a hot topic in the industry?

**Hogreve:** Classical, container-closure integrity for primary packaging containing final drug product has been an important topic in the past. But why should it be different for single-use systems used in the manufacturing of these drugs? As single-use systems are increasingly being used in all process steps of commercial manufacturing, integrity failure can have a significant impact on drug safety, availability, and costs. As a consequence, growing industry scrutiny of single-use system integrity increases the need to develop good science behind the detection of liquid leakage and microbial ingress mechanisms, and to use appropriate physical integrity-testing technologies. Sartorius Stedim Biotech developed this pioneering science and technology for higher integrity assurance.

**BioPharm/PharmTech:** How is Sartorius addressing this demand?

**Hogreve:** Sartorius has compiled a strong integrity-assurance strategy that has three pillars: consistent robustness, integrity science, and integrity-testing technologies. Consistent robustness is the basis for strong integrity assurance for single-use systems. Because testing quality into non-robust products is not an appropriate approach, it is best if quality is consistent right from the start.

Nevertheless, integrity testing is part of Sartorius’ strategy. To develop appropriate integrity-testing technologies, it is critical to understand integrity science—the failure modes, defect characteristics, and barrier properties related to the integrity of the company’s single-use systems.

Last but not least, Sartorius has developed several integrity- and leak-testing technologies to confirm the system integrity of its products, both in manufacturing as well as at the customer site.

**BioPharm/PharmTech:** You mentioned that consistent robustness is the basis for integrity assurance. What exactly do you mean by that?

**Hogreve:** As I mentioned, it does not make sense to test quality into non-robust products. Robustness becomes a critical quality attribute of a single-use system when a product range is developed according to quality-by-design principles. The final product robustness is the result...
of several ways of measuring quality, which cover the entire production chain—from testing resin properties and inspecting the films and bag chambers manufactured, to monitoring welding and assembly processes.

In addition, Sartorius performs the most stringent liquid-shipping validation in accordance with ASTM D4169 to confirm the robustness of its products under real-use and laboratory conditions. Records of this validation show that Sartorius’ Flexsafe® bags can withstand acceleration forces up to 20 g.

**BioPharm/PharmTech:** How can integrity science help to enhance the integrity of single-use systems?

**Hogreve:** Mitigating risks as part of a quality risk-management approach has become a key requirement imposed by the pharmaceutical industry and regulatory authorities. Most recently, it has led to the revision of Annex 1, “Manufacture of Sterile Medicinal Products.”

As a result of companies’ quality risk-management plans for improving process integrity, it may be required to implement an integrity-testing strategy to meet regulatory expectations and increase patient safety. This is possible only if liquid leak and microbial ingress mechanisms are thoroughly understood so that the levels of detection can be determined, which are the maximum allowable leakage limits, or MALLs. Physical integrity test methods must detect MALLs to correlate such levels of detection to leak sizes causing liquid loss and microbial ingress.

**BioPharm/PharmTech:** Is determination of the MALL required to implement meaningful integrity testing? How exactly is this determination made?

**Hogreve:** Common applications such as storage and shipping are performed under process conditions that may have an impact on the leak size, causing liquid leaks and microbial ingress. Considering storage conditions, static pressure affects the leak size. Take the example of a 500-L bag or a suspended 20-L bag with a column water height of approximately 0.7 m, which corresponds to a hydrostatic pressure of 70 mbar.

Regarding shipping conditions, the differential pressure during shipping has no impact on a flexible container, unlike on a vial. Instead, the impact results from the acceleration and/or shocks that such a bag will experience during shipping. This will affect the leak size and can be considered worst-case process conditions.

In total, four different test pressures and three different model solutions were selected to establish a theoretical model that can be used to predict the MALL under any process condition. With these parameters, a significantly large number of tests on several hundred samples have been (and are still being) performed to characterize the liquid leak and microbial ingress mechanisms on the company’s film materials. For both studies, film patches with laser-drilled holes of various leak sizes are used. Liquid leak samples are pressurized for up to 30 days under continuous visual inspection to detect any liquid leaks using indicator paper. Microbial ingress samples are challenged by aerosolization for three hours, with a challenge concentration of $10^6$ CFU/cm² *Bacillus atrophaeus*, subsequently incubated for two weeks and then visually inspected for growth. With this testing, Sartorius is able to define the MALLs for its products and consequently use them as detection limits for the company’s physical integrity testing technologies.

**BioPharm/PharmTech:** Can you give us an overview of these physical integrity testing technologies?

**Hogreve:** Sartorius is the only company that has been able to identify the MALL under process conditions and develop a technology capable of detecting the defect size that correlates to microbial ingress. Sartorius’ in-house helium integrity tester provides a sensitivity of 2 μm, and its pressure decay point-of-use integrity testing method 10 μm, so both of these values correlate to the MALL determined under process conditions in which the company’s 2D and 3D bags are used.

In addition, Sartorius provides a huge variety of point-of-use leak testing across its product portfolio. This is mainly done post installation into the final container to cover all potential gross leaks that may have been introduced during shipping, handling, and installation of the single-use system. With this, customers can confirm the system integrity right before use.
Contin. from page 25

generated (e.g., tissue-specific stem cells undergoing expansion in the culture versus directed differentiation of pluripotent stem cells into specialized cells) can be the determining factors for the choice of unit operations, production strategy, and scale up considerations for the allogeneic cell therapies."

For instance, the use of microcarrier systems versus aggregate cultures in suspension systems or the use of static two-dimensional (2D) large-scale cell-culture systems require different scale-up considerations, including the seed train strategy, the choice of bioreactor, bioreactor configurations, etc. In addition, the downstream processing steps can vary significantly depending on the product specifications and available downstream technologies, such as tangential flow filtration, counter-flow centrifugation, and continuous centrifugation methods.

Importantly, the process control strategy could be different for different cell therapy applications. "While production of large numbers of actively proliferating cells would be the primary focus for tissue-specific stem cells (e.g., mesenchymal stem cells), the control strategy for directed differentiation of pluripotent stem cells into specialized cells requires tight control of change in cell phenotype from pluripotent into multi-potent stem cells or progenitor cells and eventually fully committed cell types," Baghbaderani explains.

The fact that cell therapies are living products is a key difference when looking at cell versus gene therapies, according to Alain Lamproye, CEO of Yposkesi. "It is essential to have a sufficient amount of starting material cells that will provide a sufficient quantity of the final product. In addition, end sterilization is not possible, so the scaled expansion and production processes must guarantee sterility. For in-vivo gene therapy applications, on the other hand, the final product is a viral vector containing the gene of interest. The expansion process step is followed by clarification, purification, and final sterilization steps. The additional steps aim to increase the quality of the product, but may also reduce the quantity," he observes.

SIMILAR PROCESS DEVELOPMENT CHALLENGES

Early processes developed for cell therapies involve many manual operations and have been—according to Lamproye—created using art more than science, and lack necessary control. "To develop industrial processes," he says, "a great deal of understanding of the production process is required, starting with the demonstration of repeatability." In addition, companies must not only drastically increase manufacturing capacity, but at the same time reduce raw material (media, culture ware/bioreactors, etc.) costs to make their products accessible to more than the few people that participate in clinical trials.

"To develop a process that is repeatable and transferable to large scale is not trivial. The challenge to increasing capacity for a cell therapy process is the biological variation of cellular starting materials. Statistically relevant measurements must be repeated many times to improve process yields and reduce manufacturing cost, which adds cost and time to the development process," Lamproye observes. He points in particular to the avoidance of the use of plasmids and the development of packaging/stable cell lines as current key issues.

Reducing manufacturing costs, as well as development of scalable, automated manufacturing processes under closed systems that can reach the target quantities while maintaining the critical quality attributes (CQA) of the final products are main challenges for allogeneic cell therapies, Baghbaderani agrees. In this context, he identifies multiple important challenges:

- Modification of 2D cell-culture systems into a scalable, three-dimensional (3D) suspension-culture systems with adequate control strategies
- Completely closing processes from the starting materials to generation of final products (in particular when starting from cell-isolation steps)
- The ability to control the quality of intermediate materials during directed differentiation processes for iPSCs
- Technological limitations associated with automation of manufacturing processes
- The pace of development of automated downstream processing technologies, which have not caught up with the scale required for some of these therapies
- The fact that research-grade raw materials are often used in baseline processes for which equivalent GMP-grade materials or high-quality materials generated by qualified vendors are not available
- A lack of adequate process characterization tools and technologies

Many of these challenges apply to the development of viral-vector manufacturing processes as well. The biggest challenge, according to Baghbaderani, is the development of large-scale manufacturing processes using 3D computer-controlled suspension bioreactors with appropriate downstream processes. "Gene therapies require high productivity to meet the high dose demands. The productivity largely depends on the harvest titer and downstream process yield. Moreover, the product design at the molecular level, the producer cell line, and the production medium can all be limiting factors for harvest titer. Most importantly, appropriate analytical methods and assays are needed for implementation of proper in-process control strategies needed to achieve the desired quality and quantity of the final product," he comments.

On a big-picture level, the increased demand for viral-vector manufacturing services to support clinical trials for recombinant adeno-associated virus (rAAV)-based gene therapies has led to a shortage of production capacity. Wait times for production slots can
be 18 months or more (1). “As rAAV demand goes unmet, companies face lost opportunities, patient access to existing treatments is reduced, and development plans for new gene therapies stall,” Lamproye asserts.

COMMON VALIDATION Hurdles
One of the primary requirements for process validation is to demonstrate documented evidence that the scaled process can consistently produce the final product, meeting the CQAs based on the specified critical process parameters and critical material attributes. To achieve this goal, according to Baghbaderani, it is crucial to perform characterization and process limit evaluations of the scaled manufacturing process. “A big challenge with allogeneic applications is making sure the identity and the quality of the final product is controlled within the design parameters of the process. Lack of adequate process characterization and appropriate in-process controls due to the myriad number of process variables along with poorly defined CQAs poses significant challenges for characterization and validation of allogeneic cell therapies,” he states.

Demonstrating repeatable processes when there is biological variation due to the starting material—a key challenge mentioned for process development—also creates difficulties for process validation, agrees Lamproye. “Production engineering principles require a controlled process to utilize well-understood and consistent methods for production. The ability to generate and manufacture well-characterized batches of cells for therapeutic use requires significantly different approaches to typically implemented laboratory-based cell-culture techniques, protocols that are often labor-intensive and limited to small scale,” he says. In many cases, it is necessary to create a new process and then perform sufficient runs to generate enough data to demonstrate consistency, an effort that comes at a high cost and consumes significant resources.

As hundreds of cell and gene therapies move through clinical trials, technology suppliers race to develop suitable processes.

For gene-therapy processes, similar to allogeneic processes, poorly defined CQAs for the final product and inadequate process characterization (potentially due to the numerous process variables and lack of appropriate in-process monitoring and controls) are the main challenges for process validation, according to Baghbaderani. Precise in-process monitoring of complex parameters (metabolite concentrations, pH, oxygen and carbon dioxide, number and viability of the cells) within the bioreactor is necessary to gather the data needed to enable effective scale up of upstream processes, Lamproye adds. In many cases, he notes that the need to develop, validate, and implement novel analytical techniques is an added challenge.

SOME NEW TECH IS HELPING
Overall, the pace of technology development is not in harmony with the growth in the number of cell and gene therapies moving through the clinic, according to Baghbaderani. There are, however, some new technologies that have been helpful with respect to the development of robust and reproducible manufacturing processes.

For cell-therapy applications, Baghbaderani points to the Cocoon Platform from Lonza, an automated GMP-in-a-box concept for patient-scale cell therapy manufacturing, other iPSC platform technologies that allow the generation and characterization of high-quality pluripotent stem cells, and new downstream technologies specific for processing cell therapy products, such as Acoustic Cell Processing (FloDesign Sonics), the Sepax Cell Separation System (GE Healthcare), and the LOVO system (Fresenius Kabi).

For Lamproye, a key advance for cell-therapy manufacturing has been the development of bioreactors that enable 3D production using systems that were originally designed for 2D flatware. “The ability to transfer production systems for adherent cells to bioreactors aids in the scale up of these processes. Bioreactors can be used as closed systems and provide the opportunity to scale up to larger volumes. And importantly, the science of bioreactor scaleup is better understood and generally requires few process changes,” he explains.

For gene therapies, advances in analytical techniques designed to address the needs of these complex products, such as digital droplet polymerase chain reaction, flow cytometry, and high-performance liquid chromatography, have enabled more consistent and accurate measurement of quality attributes, observes Baghbaderani.

SCALE OUT OF AUTOLOGOUS THERAPIES
With autologous therapies, scaling studies actually focus on scale-out, with the implementation of multiple parallel processes for manufacturing of final products, each of which uses material from one patient. “To produce products for large numbers of patients would require running of hundreds to thousands of parallel processes in a cleanroom environment, which is not feasible. It will be necessary to develop an automated closed system with a minimal footprint that can be placed in a cleanroom space yet still maintain material traceability.”

Contin. on page 42
ABSTRACT
Gold nanoparticles (AuNPs) are important components for biomedical applications and are widely employed for diagnostics and therapeutics. Nanoparticles are mainly synthesized through chemical and physical methods, which are often costly and potentially harmful. Synthesis of nanoparticles using plants, however, is less toxic and more effective. Recently, researchers have been focusing on green synthesis of AuNPs. This study aims to use plant-leaf extract for the green synthesis of AuNPs and to evaluate their antibacterial and antioxidant activity. The results indicated that AuNPs can be synthesized using a simple method with extracts from Adiantum capillus veneris (ACV) and Pteris quadriureta (PQ) leaves. The characterization of the AuNPs was done by ultraviolet-visible spectroscopy, powder X-ray diffraction, Fourier transform infrared spectroscopy, and energy dispersive X-ray spectroscopy. The nanoparticles of ACV and PQ were seen at the wave length of 573 nm and 520 nm, respectively. The nanoparticles of both ACV and PQ leaves extract showed antioxidant, antibacterial, and antifungal activities. ACV nanoparticles showed increased antioxidant and antimicrobial activity compared to PQ. Taken together, the results reveal that the AuNPs synthesized from leaves of ACV and PQ possess antioxidant and antimicrobial activity.
culosis, and cardiovascular diseases, based on their biocompatibility and non-cytotoxicity (21–25).

In a previous study, the researchers of this study provided compelling evidence for the antioxidant and antimicrobial activities of the crude extracts of *Adiantum capillus veneris* (ACV) and *Pteris quadriureta* (PQ) (26). In the present study, researchers synthesized the AuNPs from the extract of ACV and PQ leaves by a green biological route and characterized them using ultraviolet-visible (UV-Vis) spectroscopy, energy dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR) analysis. Furthermore, the antioxidant and antimicrobial properties of the synthesized nanoparticles were examined.

**MATERIALS AND METHODS**

**Collection, identification, and processing of plants**
The leaves of ACV and PQ plants were collected from Kodaikanal hills, Tamil Nadu, India. The plants were identified by Regional Plant Resource Centre, Odisha Biodiversity Board (No. 2175). The plant leaves were washed thoroughly thrice with distilled water, shade dried for five days, and blended into a fine powder. The leaf powder was sterilized at 121 °C for 15 min. Next, 20 g of powder was mixed with 200 mL of distilled water and kept in a boiling water bath at 60 °C for 10 min. The extracts were filtered (Whatman filter paper No. 1), and the filtered extract was stored in the refrigerator at 4 °C for further studies.

**Synthesis of AuNPs and visual analysis**
For the biosynthesis of AuNPs, 1.5 mL of plant extract powder was mixed with 30 mL of auric chloride solution (1 mM/mL) and incubated at 28 °C for 24 h. The color change in reaction mixture (auric chloride solution + leaf extract) was recorded through visual observation. The bioreduced gold nanoparticle solution was filtered (Whatmann No.1 filter paper), and the bioreduction of pure gold ions was observed at different time intervals by monitoring the UV-Vis spectrum of the solution between 500 nm to 600 nm using a spectrophotometer (Thermo-Biomate 3 UV-visible spectrophotometer).

**X-ray diffraction measurements**
The completely bioreduced sample was concentrated in an oven at 50 °C, and the concentrated solution was then centrifuged at 6000 rpm for 15 min. The obtained pellet was washed and redispersed in deionized water. The dried mixture of AuNPs was collected to determine the formation of AuNPs by XRD operated at a voltage of 30 kV and a current of 30 mA with Copper K-alpha radiation in a θ–2θ configuration.

**Determination of crystalline size**
Average crystallite size of gold was calculated using Scherrer's formula (Equation 1):

\[
D = \frac{k \lambda}{\beta \cos \theta} \\
\text{[Eq. 1]}
\]

Where \(D\) = average crystallite size, \(k\) = constant, \(\lambda\) = X-ray wavelength, \(\beta\) = angular full width at half maximum (FWHM) of the XRD peak at the diffraction angle, and \(\theta\) = diffraction angle.

**FTIR analysis**
FTIR spectra of the aqueous leaf extract and AuNP samples were analyzed by FTIR spectroscopy (Hitachi). The FTIR analysis was performed with potassium bromide (KBr) pellets and recorded in the range of 400 cm\(^{-1}\)–4000 cm\(^{-1}\). The various modes of vibrations were identified and assigned to determine the different functional groups present in the samples.
EDX analysis
Scanning electron microscope (SEM) (JSM-5800 LV, JEOL) was used to determine the shape of developed nanoparticles. Samples were prepared by depositing a drop of colloidal solution on an aluminium grid sample holder and drying at room temperature. Elemental composition of the sample was analyzed with energy dispersive analysis of EDX coupled to the SEM.

DPPH radical scavenging assay
Free radical scavenging activity of the nanoparticles was analyzed by a 2,2-diphenyl-1-picryl-hydrayl-hydrate (DPPH) assay. Different concentrations (10–80 μg/mL) of AuNPs were added in equal volume to 0.1 mM methanolic DPPH solution. After 30 minutes of incubation at room temperature, absorbance of the samples was read at 517 nm. Ascorbic acid was used as a standard. The experiment was repeated in triplicate, and the DPPH scavenging activity was calculated by percent inhibition (Equation 2):

\[
\text{Inhibition percent} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100
\]  
\[\text{Eq. 2}\]

where \(A_0\) is the absorbance of the control and \(A_1\) the absorbance of the NPs solution.

Hydroxyl radical assay
The hydroxyl radical scavenging activity of AuNPs of ACV and PQ was evaluated by hydroxyl radical assay (27). The reaction mixture containing dilution series from 10 μg/mL to 100 μg/mL of AuNPs was incubated with deoxyribose (3.75 mM), hydrogen peroxide (H\(_2\)O\(_2\)) (1 mM), iron (III) chloride (FeCl\(_3\)) (100 μM) in phosphate buffer (pH 7.4). The reaction was terminated by thiobarbituric acid (1 mL; 1% w/v) and trichloroacetic acid (1 mL; 2% w/v) and then heated in a boiling water bath for 15 min. Pink chromagen was formed, which eventually resulted in the formation of thiobarbituric acid reactive substances (TBARS). The content was cooled, and absorbance of mixture was measured at 535 nm against a blank. The percent inhibition of hydroxyl radical generation was calculated using Equation 3:

\[
\text{Inhibition percent} = \left(\frac{C - T}{C}\right) \times 100
\]  
\[\text{Eq. 3}\]

where \(C\) is absorbance of the control and \(T\) is the test samples.

Superoxide radical-scavenging assay
The superoxide radical scavenging activity of AuNPs from ACV and PQ was evaluated by superoxide radical scavenging assay. The reaction mixture was made up of 3 mL (50 mM sodium phosphate buffer [pH 7.6], 20 μg riboflavin, 12 mM ethylenediaminetetraacetic acid [EDTA]), 0.1 mg nitro blue tetrazolium (NBT), and 100 μL sample solution. The reaction mixture was incubated for 90 s, and, immediately after illumination, the absorbance was measured at 590 nm. The entire reaction assembly was enclosed in a box lined with aluminium foil. Identical tubes with reaction mixture kept in the dark served as blanks. The percentage inhibition of superoxide
anion generation was calculated as shown in Equation 4:

\[
\% \text{ inhibition} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100
\]

[Eq. 4]

where \(A_0\) is the absorbance of the control, and \(A_1\) is the absorbance of the sample extract and standard (28).

**Measurement of hydrogen peroxide scavenging activity**

The ability of AuNPs to scavenge \(\text{H}_2\text{O}_2\) was determined according to the method of Ruch et al. (29) with slight modifications. The mixture was made up of different concentrations of AuNPs (10–80 \(\mu\)g/mL), and butylated hydroxytoluene (BHT) was mixed with 2.4 mL of phosphate buffer (0.1 M, pH 7.4) and 0.6 mL of \(\text{H}_2\text{O}_2\) solution (40 mM). Then the mixture was vortexed and incubated at room temperature for 10 min. At the end of incubation, the concentration of \(\text{H}_2\text{O}_2\) was determined by absorbance at 230 nm against a blank solution containing phosphate buffer without \(\text{H}_2\text{O}_2\). The \(\text{H}_2\text{O}_2\) scavenging ability was calculated using the formula as described for DPPH assay (Equation 2).

**Antimicrobial activity**

Bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteric*, *Staphylococcus aureus*, and *Bacillus subtilis* and fungi such as *Trichophytonrubrum*, *Scedosporiumapiospermum*, *Aspergillus fumigates*, *Aspergillusniger*, and *Aspergillusflavus* were collected and clinically isolated. Each bacterial strain was suspended in a nutrient broth and incubated for 18 h at 37 °C. Nutrient agar (NA) and potato dextrose agar (PDA) were used for the study of antibacterial activity and antifungal activity, respectively. The nutrient-broth-cultured bacteria were spread over a NA plate, whereas a 24-h cultured fungi was spread on PDA by using cotton swab. A 5-mm disc was dipped in each nanoparticle solution, including a positive control solution, such as ampicillin and itraconazole (10 \(\mu\)g), for bacteria and fungi, respectively, and placed on the swabbed agar plate. Each disc absorbed 15 \(\mu\)l of sample, which is made up of 50 mg/mL and 100 mg/mL concentrations. The plates were then incubated at 37 °C for 24 h for bacterial pathogens and 72 h for fungal pathogens. The antimicrobial activity was evaluated by measuring the diameter of the inhibition zone.

**Statistical analysis**

The data of various analyses were expressed as mean ± standard deviation. All tests were carried out in triplicate to improve the accuracy.

**RESULTS**

**UV analysis**

The formation of purple color (Figure 1C) after mixing the plant extracts with auric chloride (HAuCl4) solution showed the presence of AuNPs. Figure 1A shows the HAuCl4 solution, Figure 1B is the aqueous extract solution of the plant, and Figure 1C is the gold nanoparticle-synthesized solution. Figure 2 shows the UV-Vis absorption spectra of synthesized AuNPs. The UV-Vis analysis (Figure 2) also confirmed the formation of AuNPs at a wavelength of 573 nm and 520 nm of ACV and PQ plant extracts, respectively.

**XRD analysis**

The X-ray diffraction pattern (XRD) revealed that AuNPs of ACV and PQ are crystalline in nature. The Debye–Scherrer’s equation was used.
to calculate the size of the AuNPs on the basis of the FWHM of the (111) Bragg’s reflection arising from the diffractograms. The intensity of the peaks of ACV of (111) at 38.91° diffraction was much stronger than those peaks of (200), (220), and (311) at 45.02°, 65.34°, and 78.24°, respectively, and the intensity of the peaks of PQ of (111) at 38.92° diffraction was much stronger than those peaks of (200), (220), and (311) at 45.08°, 65.36°, and 78.26°, respectively. The Bragg reflections of lattice planes showed the face-centered cubic structures for gold, and the broadening of Bragg’s peaks indicates the formation of nanoparticles. A few additional and unassigned peaks were also observed, which resulted because of bio-organic compounds or proteins in the nanoparticle during the synthesis. These unassigned peaks did not interfere with the Bragg reflection peaks but showed the synthesis of AuNPs, and they played a role in the stabilization of AuNPs (Figure 3).

FTIR analysis
FTIR analysis was done to detect the possible biomolecules which play a role in the reduction of AuNPs following the stabilization-capping. Figure 4 shows the FTIR spectra of the ACV and PQ leaf extract and AuNPs. We found strong bands at 3425 cm⁻¹, 2920 cm⁻¹, 2382 cm⁻¹, 1626 cm⁻¹, and 1401 cm⁻¹ of ACV extract along with a few weak bands, including 1077 cm⁻¹ and 610 cm⁻¹. In the case of PQ, it showed strong bands at 3416 cm⁻¹, 2929 cm⁻¹, 1635 cm⁻¹, 1396 cm⁻¹ and weak bands at 1082 cm⁻¹ and 691 cm⁻¹. Bands at 3425 cm⁻¹ and 3416 cm⁻¹ correspond to O–H stretching modes from alcohol and phenol groups, while bands at 2920 cm⁻¹ and 2929 cm⁻¹ belong to saturated systems alkanes. Bands at 2382 cm⁻¹ arise because of the stretching of C–C of the alkyne group, and bands at 1626 cm⁻¹ and 1635 cm⁻¹ arise because of the C=O functional group of amide in stretching mode. Bands at 1401 cm⁻¹ and 1396 cm⁻¹ belong to the methylene group, whereas the weakest bands, including 1077 cm⁻¹ and 1082 cm⁻¹ as well as 610 cm⁻¹ and 691 cm⁻¹ correspond to the C–O–C functional group and arenes group, respectively.

Energy dispersive X-ray analysis
EDX analysis confirmed the presence of AuNPs of ACV and PQ plant extracts, which accounted for 41.85% and 48.23% by weight of ACV- and PQ-analyzed samples, respectively. A strong and clear peak of gold atoms was observed in the spot-directed EDX spectrum of all the AuNPs of both the plant extracts. In the case of ACV, oxygen (O), manganese, potassium (K), chloride (Cl), and calcium atoms were indicated by the weaker signals. In the case of PQ, O, K, silicon, and Cl atoms were also shown by the weaker peaks, which may have resulted from X-ray emissions from proteins/enzymes present in the biomolecules, which can cause capping of the AuNPs (Figure 5).

Antioxidant activity
The ACV and PQ nanoparticles exhibited free radical scavenging activities, with the ACV nanoparticles exhibiting the highest activity, followed by PQ nanoparticle activity. At concentrations of 10 μg/mL to 200 μg/mL, the scavenging activities of ACV were 35% to 91%, while the scavenging activities of PQ were 22% to 81% (Figure 6A). Further, the superoxide radical scavenging activity of the ACV nanoparticles was found to be significantly higher than that of the PQ nanoparticles (Figure 6B).
Antioxidant potential of the ACV and PQ nanoparticles was further estimated using hydroxyl radical scavenging activity. It was observed that the scavenging activity of nanoparticles of ACV and PQ was increased from 31% to 85% and 20% to 74%, respectively, at concentrations of 10 μg/mL to 200 μg/mL (Figure 6C). The scavenging ability of nanoparticles of ACV and PQ on H$_2$O$_2$ is shown (Figure 6D). The nanoparticles of ACV and PQ were capable of scavenging H$_2$O$_2$ in a dose-dependent manner.

**Antimicrobial activity**

Tables I and II show the antibacterial and antifungal activities of nanoparticles of ACV and PQ leaves. Two concentrations (50 mg/mL and 100 mg/mL) of nanoparticles were tested against five different bacteria (B. subtilis, E. coli, P. aeruginosa, S. enteric, and S. aureus) and five different fungi (A. niger, A. fumigates, A. flavus, T. rubrum, and S. apiospernum). There was a significant increase in the zone of inhibition as the concentration of AuNPs increased (Figures 7A and 7B).

**DISCUSSION**

Medicinal plants are in demand because of their biological properties and bioactive compounds, which are known to act against various diseases (30–32). The green synthesized nanoparticles have attracted global attention because of their unique properties and because they have fewer side effects (33). The present study suggests that nanoparticles of ACV and PQ leaves possess antioxidant, antibacterial, and antifungal activities that can be therapeutically beneficial.

The presence of gold nanoparticles was confirmed by change of color from the reduction of Au$^+$ to Au$_0$ ions at altered time intervals. The UV-Vis spectra showed strong peaks for ACV extracts at 573 nm and for PQ extracts at 520 nm. The formation of these strong, broad peaks has been found for various AuNPs with sizes ranging from 2 nm to 100 nm (34). Jayaseelan et al., (2013) has reported that aqueous extract of Abelmoschus esculentus seeds showed surface plasmon resonance at 536 nm (35).

In XRD analysis, strong peaks of both the nanoparticles were observed at range of 2θ values of (Figure 5). Energy dispersive X-ray spectroscopy (EDX) analysis of gold nanoparticles (AuNPs) produced using aqueous plant extracts, A) Adiantum capillus veneris (ACV) and B) Pteris quadriureta (PQ). In all cases, peaks are labeled. Gold is detected in all, consistent with the formation of AuNPs, while the other elements come from trace elements in the extracts.

Table I. Anti-bacterial activity of Adiantum capillus veneris (ACV) and Pteris quadriureta (PQ) gold nanoparticles. Values are means of triplicate determinations (n=3) ± standard deviation. * (p<0.05) significantly different from antibiotic.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Antibiotic (zone of inhibition in mm)</th>
<th>ACV</th>
<th>PQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>24.00</td>
<td>17.00</td>
<td>16.00</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>26.00</td>
<td>21.00</td>
<td>17.00</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>22.00</td>
<td>18.00</td>
<td>16.00</td>
</tr>
<tr>
<td>Salmonella enteric</td>
<td>21.00</td>
<td>16.00</td>
<td>16.00</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>39.00</td>
<td>19.00</td>
<td>18.00</td>
</tr>
</tbody>
</table>
38.91, which corresponded to the Bragg’s reflections of (111), (200), (220), and (311). FTIR analysis of the ACV and PQ leaves’ extracts indicated that the carboxyl groups (C=O), hydroxyl groups (OH), and amine groups (N-H) of both plant extracts are mainly involved in the reduction of Au+ nanoparticles to Au0 nanoparticles by acting on the C–C bond in the alkyne group. Similar results were described by other studies, in which it was reported that the vibrational bending of the peaks may have occurred from the presence of compounds in the plant extracts, including flavonoids, terpenoids, alkaloids, and soluble proteins. The presence of these compounds in the extracts may also cause the stabilization of gold nanoparticles (36). The EDX profile of AuNPs of ACV and PQ showed strong signals for gold atoms and weak signals for O, K, Si, and Cl atoms. The presence of these characteristic compounds in the extracts may have contributed to the observed antimicrobial activity.

**Table II.** Anti-fungal activity of *Adiantum capillus veneris* (ACV) and *Pteris quadriureta* (PQ) gold nanoparticles. Values are means of triplicate determinations (n=3) ± standard deviation.

* (p<0.05) significantly different from antibiotic.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Antibiotic (zone of inhibition in mm)</th>
<th>ACV</th>
<th>PQ</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus niger</em></td>
<td>24.00</td>
<td>20.00</td>
<td>16.00</td>
</tr>
<tr>
<td><em>Aspergillus fumigates</em></td>
<td>25.00</td>
<td>16.00</td>
<td>15.00</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>24.00</td>
<td>20.00</td>
<td>15.00</td>
</tr>
<tr>
<td><em>Trichophyton rubrum</em></td>
<td>29.00</td>
<td>17.00</td>
<td>14.00</td>
</tr>
<tr>
<td><em>Scedosporium apiospermum</em></td>
<td>30.00</td>
<td>16.00</td>
<td>15.00</td>
</tr>
</tbody>
</table>
Peaks of gold nanoparticles shows the effective combination of polyphenolics on the outer surface of the gold nanoparticles (37–39).

DPPH and superoxide radical scavenging activity are widely used for testing preliminary radical scavenging activity of compounds or nanoparticles and provide easy and rapid evaluation. In the present study, the synthesized AuNPs exhibited potential free radical scavenging activity against both DPPH and superoxide radicals. The presence of polyphenolic compounds such as flavonoids, flavonols, proanthocyanidin, and phenolics in plants have been reported to have strong antioxidant activities, which help to protect cells against oxidative damage by free radicals (40). It was reported that the antioxidant activities of plant extracts were enhanced by conversion into gold nanoparticles (41).

The hydroxyl radical formed in the Fenton reaction in the presence of reduced transition metals is known to be the most reactive of all the reduced forms of dioxygen and is capable of damaging almost every molecule found in living cells (42). An increase in hydroxyl radical scavenging activities with increasing concentrations of nanoparticles was observed in this study. Medhe et al. (2014) reported that dihydroxy flavone-coated nanoparticle showed increased hydroxyl radical scavenging activity (43). In addition, the gold nanoparticles of ACV and PQ exhibited increased H₂O₂ scavenging activity. H₂O₂ scavenging by phytochemicals may be attributed to the donation of electrons to H₂O₂, thus neutralizing it to water (44).

AuNPs synthesized by natural compounds extracted from plants are gaining importance. The nanoparticles of ACV and PQ were very effective in inhibiting microbial growth, which may be due

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Figure 7. A. Anti-bacterial and B. anti-fungal activity of aqueous extracts of *Adiantum capillus veneris* (ACV) and *Pteris quadriureta* (PQ) leaves; a. control, b. positive control, c. gold nanoparticles.
to the presence of sterols and secondary metabolites. AuNPs were found to be toxic for microbial organisms (45–48). Sreelakshmi et al. (2011) reported that AuNPs synthesized by using natural honey showed increased antimicrobial activity (49). Zhang et al. (2016) reported that green-synthesized AuNPs show efficient antibacterial activity compared to chemically synthesized AuNPs, which showed nearly no antimicrobial activity (50–51).

The present study demonstrates an eco-friendly and low-cost method for biosynthesis of AuNPs from ACV and PQ. This method of reduction used here is simple, easy to perform, inexpensive, and eco-friendly as a substitute to chemical synthesis. The biosynthesized AuNPs showed antioxidant and antimicrobial activities, demonstrating their utility in biomedical applications. Testing of various other biological applications of these nanoparticles is warranted.

ACKNOWLEDGEMENTS
We acknowledge Sukanya and A. Rajanandhini for their help in conducting this work.

REFERENCES
Getting to the Root of Quality Problems

Focusing on symptoms instead of root causes locks teams into a corrective, rather than preventive, mindset.

AGNES SHANLEY

The philosopher George Santayana once wrote, “Those who fail to learn from the past are condemned to repeat it.” This is clearly seen in the way that some pharmaceutical manufacturers approach root cause analysis (RCA) and corrective action and preventive action (CAPA).

Both RCA and CAPA are closely intertwined. For example, tracking and trending complaints allows companies to identify recurring problems that may not be caught during inspections on the manufacturing floor, says Kim Jackson, product manager at MasterControl, a vendor of quality management and CAPA software. It also allows them to determine the true severity of specific problems or failure modes, she says. Furthermore, CAPA effectiveness checks ensure that RCA investigations are sufficiently robust, says pharma quality consultant Ajay Pazhayattil.

Both concepts are crucial for establishing continuous improvement and a true culture of quality, and achieving the goals established by International Council for Harmonization (ICH) Q12. “Efforts always pay off because they prevent supply disruptions and resulting revenue loss,” says Pazhayattil. Addressing them incorrectly, however, can lead to product quality failures as well as noncompliance with current good manufacturing practices (CGMPs). In some cases, particularly for over-the-counter (OTC) drugs, the problems highlighted in warning letters had already been pointed out previously in FDA Form 483s (1), suggesting a need for more senior management involvement and greater investment in CGMP compliance. Inspectors pointed to inadequate validation and conformance to written procedures, as well as deficient RCA and CAPA. In some cases, root causes for batch or product testing failures were either insufficiently investigated or not probed at all (2,3).

UNDERSTANDING THE COST OF POOR QUALITY

Experts see a number of reasons for this situation, including the need for a more rigorous approach to risk assessment and a better understanding of the cost of poor quality. “If we as an industry could step up and own the cost of poor quality, if we could measure it, we’d be horrified at how much we pay, not just for failing to solve issues but for repeatedly solving the same ones,” says Nuala Calnan, principal of the consulting firm BioPharm Excel and professor at the Technical
University of Dublin. “The number of personhours and CAPAs that arise from these investigations is eyewatering. We don’t add them all up and have a trackable number (i.e., that’s how much it cost us not to get to root cause and to keep on failing),” she says.

But there has also been a lack of good RCA training at many pharmaceutical manufacturers. In October 2019, the International Society for Pharmaceutical Engineers (ISPE) and the Parenteral Drug Association (PDA) launched their joint Quality Culture guidance series with a module devoted to best practices for RCA (4), in an attempt to address this problem. Its goal is to help shift the industry’s focus from compliance to prevention. “Pharma companies typically cover fundamentals (e.g., how to use tools such as Five Whys and Ishikawa fishbone diagrams), but teaching people how to use tools and templates isn’t training them to look at the underlying science behind RCA decision making and why it is so important,” says Calnan, who is also coleader of ISPE’s quality culture initiative and one of the authors of the new RCA module.

**BETTER RCA TRAINING NEEDED**

Advanced training in the techniques and critical thinking required for investigators, and application of proven RCA tools such as mapping, brainstorming, cause-and-effect, and Five Whys are crucial to improving the state of RCA in the industry, says Pazhayattil. “It’s important not to get stuck on using the same methodology each time, since different problems will call for different solutions,” suggests Marzena Ingram, a senior pharma quality manager who comments as an industry professional rather than on behalf of her company.

Impeding a better approach to RCA at many companies is an ingrained focus on regulatory compliance and being inspection-ready. “Many quality departments must focus on feeding the compliance system rather than considering quality as a broader responsibility. Often, we’re being driven to close out investigations within 30 days because compliance metrics tell us we need to do that, so we’re not addressing root cause,” says Calnan. In some cases, companies may over-respond to smaller quality problems by launching too many full-scale RCA investigations, says Jackson. “When a company goes into full alert, all-hands-on-deck mode for every issue that arises, not only does time get wasted, but employees become jaded and less likely to do due diligence when a real issue presents itself,” she says. “Taking a risk-based approach to escalations and root cause investigations saves efforts for issues that truly pose a risk to patient health and safety,” she says. Jackson suggests that each quality event be investigated first at a lower level to determine the most likely cause, and then evaluated for risk, says Jackson.

Another fatal flaw in many RCA programs is that the approaches typically address only part of the problem, the symptoms of the problem, or its direct cause, rather than the fundamental reason why it happened. For example, operator error, which is often cited as the reason for quality problems, usually shows that the system of controls in place for the process and product has failed, rather than any single individual, Calnan says. In other cases, the reason behind the direct cause for one batch’s failure (e.g., a product or labeling mix-up) may be solved without considering the series of events that caused the mix-up to occur in the first place. In all these cases, companies wind up cutting and pasting familiar causes of failures, but theoretical assumptions should never bias an investigator,” he says. For example, an investigator may unconsciously or consciously, focus on the area that he or she is more familiar with (e.g., process engineering or analytical methods). “Generating sound supporting proof is critical to confirming root causes and developing a science- and data-driven investigation method,” he says.

“Coming into an investigation with a biased opinion, even if you are fairly sure you’re right, is a surefire way to miss the opportunity to investigate with an open mind,” says Ingram. “No ideas are bad ideas, so it’s best for teams to brainstorm, throw all possibilities up on the board and then rule out, systematically and with proper justification,” she says.

**DEVIL’S ADVOCATES NEEDED**

Calnan also sees an overemphasis on consensus-building as impeding the effectiveness of pharma risk management and RCA. With group efforts such as failure modes and effects analysis (FMEA), which require teams to agree on a numerical value to assign to each specific risk, there may be a failure to invite diversity of opinion or different perspectives.

“Consensus [can become] the enemy of good critical thinking and risk management,” she says. When teams are analyzing a quality problem, Calnan suggests that one or more members play devil’s advocate and ask for data or other evidence to support any hypotheses. “In some cases, companies may be pushing for agreement way too soon, before they’ve spent enough time or applied any real rigor to identifying the real issues that are causing the problem,” she says.

“There is very little in pharma’s processes that gets us back out there, onto the lab or manufacturing floor, to shake down the cursory one liner that was given as the reason for the problem. As a result, companies often solve for the wrong problem. When
they haven’t even identified the right problem, how can they get to its root cause?” Calnan asks.

Another obstacle to improvement is the fact that the industry doesn’t typically view failures as an opportunity for learning, says Calnan. “All too often, we have to classify failures, include them in an investigation report, and apply a CAPA to them to move them off our desks so that we can get to the next round of firefighting,” she says.

TRENDING TO PINPOINT SOURCES OF VARIABILITY

Trending should be done regularly, as suggested by FDA’s revised process validation guidance, to find where sources of variation and potential risk and failure are developing, she says. Market complaints, stability data trends, and multivariate analysis of critical process parameters and critical quality attributes are all sources of data for continuous quality improvement, says Pazhayattil.

In addition to evaluating risk for individual events, monitoring should be put in place to help identify recurring trends that should be investigated, says Richards. If an issue is recurring, or multiple events point to the same recurring cause, the recurrence can be investigated, and a risk assessment performed on the trend. Based on that, a company could continue to monitor, setting a threshold of acceptance, or initiate a full root cause investigation and CAPA, she says.

When a full root cause investigation is warranted, having a standard methodology can help streamline the analysis, she says. For example, the use of Five Whys provides structure to the investigation and can help contain its scope while ensuring thoroughness.

Staffing cross-functional teams offers an opportunity to ensure that the right skill sets are available for RCA. “I am seeing more such teams in action today, but many of them still take an ‘us vs. them’ approach,” says Calnan. This often comes out most clearly in the interpretation of such phrases as ‘the quality department is responsible for closing out the investigation.’ While not meant as an excuse to pass the buck, this phrase often results in quality executives having to develop solutions without enough input and insight from the team, including insights from those much closer to the problem,” says Calnan.

“The belief that the responsibility to assess and address root cause is ‘someone else’s problem’ is a typical pitfall in pharma RCA programs,” says Ingram. “All departments, even those that may not have direct involvement, should play a role in identifying, analyzing, and effectively solving the problem,” she says.

Training and knowledge management can also be an obstacle in some companies, where subject matter experts (SMEs) are almost empowered not to share knowledge. “SMEs need to see themselves as knowledge stewards and trainers for the next generation, and essential to creating a learning organization,” says Calnan.

NEED FOR LEADING METRICS

Beyond training and procedures, pharma’s quality metrics themselves must change if RCA and quality programs are to improve, Calnan says. She points to a need for leading (rather than lagging) metrics that are aligned with overall patient and business priorities. One example, she says, would be measuring the ratio of preventive actions to corrective actions within CAPA systems, and setting a target to move performance to the next level.

In addition, Calnan says, recurring deviations need to be correctly coded and accounted for. Currently, most companies don’t code errors in a transparent way that would make it easy to see when they recur. “They’re looking for the exact same error to happen on the exact same line or process, rather than asking whether there are common root causes and coding those causes appropriately,” she says. For example, an organization can have one problem in a lab and another on the manufacturing floor that may seem very different yet share the same root causes.

A number of technologies are available that promise to improve the way pharma handles RCA and to make the process easier. More powerful data analytics approaches are already being used to allow data to be drawn from disparate databases to be used for risk analysis and RCA, says Calnan. “Technology can also be leveraged to gather failure modes and occurrence data to better inform risk assessments, preventing unnecessary RCA activities when a simple mitigation would suffice,” says Jackson.

Machine learning and artificial intelligence (AI) tools (e.g., predictive modeling for equipment maintenance, manufacturing process control, and real-time release) are also emerging, says Pazhayattil, who is currently working on research into AI in pharmaceutical manufacturing with CalSouthern. However, it will be a long time before these technologies are found routinely on every pharmaceutical manufacturing plant floor, says Calnan.

Although modern equipment (e.g., filling equipment that incorporates automation and isolators) can help improve overall operations, Calnan believes that it is not essential to preventing failures. In addition, she says, doing RCA correctly needn’t be expensive. “As an industry, we need to get on with real training. People need to understand the science behind failure, and to understand the differences as well as the connections between risk and failure,” she says.

REFERENCES

yet still maintain material traceability (including chain of custody). Such a strategy would help reduce the overall cost of goods, making the process more viable for commercial manufacturing and ultimately offering more affordable treatments,” Baghbaderani asserts.

Any production system that is used must also ensure that processes are robust enough to enable the use of raw/starting materials with significant variability, adds Lamproye. “For instance, the variation of transplanted CD34+ cells/kg can be up to four orders of magnitude around the median for autologous purposes. Strategies for autologous cell and gene therapy production must therefore be sufficiently robust to enable the development and implementation of effective in-process controls,” he states.

THE PROCESS IS THE PRODUCT

Although there are common unit operations and similarities between manufacturing processes for cell and gene therapies, every manufacturing process is fundamentally different and unique due to the number of process variables involved in each process, the definition of the relevant CQAs, and the fact that characterization tools may be lacking, according Baghbaderani. “It can therefore be argued that the process is the product to a large extent, and it is crucial to follow best practices during early development studies and carry out proper risk assessments for each process,” he remarks. One general approach to minimize variabilities and manufacturing risks is to minimize suboptimal, open, manual unit operations and replace 2D cell-culture systems with computer-controlled, 3D suspension bioreactors.

The focus for many manufacturers, whether innovators or contract development and manufacturing organizations (CDMOs), is on automation. “Many CDMOs are now integrating sufficiently flexible production workflows by utilizing automated systems. This approach can potentially enhance process and product standardization, provide effective tracking of processes and in-process controls, and lead to higher throughput and simpler processes,” Lamproye observes. Automation and implementation of in-process controls and in-process monitoring technologies during early development, adds Baghbaderani, can significantly prevent future delays and manufacturing or commercial failures for cell and gene therapy products.

REFERENCE

NC STATE UNIVERSITY
Program manager opportunity

The Biomanufacturing Training and Education Center (BTEC) at NC State University, located in Raleigh, NC, seeks a program manager (position #00107838) to lead the new 5-year AIM-Bio project, a collaborative program between NC State and the Technical University of Denmark (DTU). To address key challenges facing the biopharmaceutical industry, AIM-Bio (go.ncsu.edu/aim-bio) will create an international enterprise focusing on bioprocess research and development and workforce education and training. The individual hired will interact with faculty and staff at both NC State and DTU and play a key role in managing all areas of the program: development and implementation of short courses, research and development projects, and international exchanges. For additional information, including position requirements, see go.ncsu.edu/aim-bio-mgr.

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