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AACR-IASLC Joint Conference on Molecular Origins of Lung Cancer
Co-Chairpersons: Roy Herbst, Elisabeth Brambilla, Pasi Jänne, and William Pao
January 6-9, 2014
San Diego, CA

AACR-Prostate Cancer Foundation Conference on Advances in Prostate Cancer Research
Co-Chairpersons: Arul M. Chinnaiyan, William G. Nelson, June M. Chan, and Jonathan W. Simons
January 18-21, 2014 • San Diego, CA
Advance registration deadline: Monday, December 9

Cancer Susceptibility and Cancer Susceptibility Syndromes
Co-Chairpersons: Alan D. D'Andrea, Phillip A. Dennis, and Pier Paolo Pandolfi
January 29-February 1, 2014 • San Diego, CA
Advance registration deadline: Monday, December 9

RAS Oncogenes: From Biology to Therapy
Co-Chairpersons: Frank McCormick, Dafna Bar-Sagi, and Channing J. Der
February 24-27, 2014 • Lake Buena Vista, FL
Abstract submission and award application deadline: Friday, December 6
Advance registration deadline: Monday, January 13

Cellular Heterogeneity in the Tumor Microenvironment
Co-Chairpersons: Mary Helen Barcellos-Hoff, Michele De Palma, and M. Celeste Simon
February 26-March 1, 2014 • San Diego, CA
Abstract submission and award application deadline: Monday, December 16
Advance registration deadline: Monday, January 13

AACR Annual Meeting 2014
Chairperson: Scott W. Lowe
April 5-9, 2014
San Diego, CA

Pancreatic Cancer
Co-Chairpersons: Dafna Bar-Sagi, David A. Tuveson, Christine Iacobuzio-Donahue, Alec Kimmelman, and Andrew M. Lowy
May 18-21, 2014
New Orleans, LA

Targeting PI3K/mTOR Networks in Cancer
Co-Chairpersons: Lewis C. Cantley, Jose Baselga, Joan S. Brugge, Brendan J. Manning, and Malte Peters
September 14-17, 2014
Philadelphia, PA

Melanoma
Co-Chairpersons: Levi A. Garraway, Keith T. Flaherty, and Suzanne L. Topalian
September 20–23, 2014
Philadelphia, PA

13th Annual International Conference on Frontiers in Cancer Prevention Research
Program Committee Chairperson: Phillip A. Dennis
September 28-October 1, 2014
New Orleans, LA

Tumor Immunology
Co-Chairpersons: Robert H. Vonderheide, Nina Bhardwaj, Stanley Riddell, and Cynthia L. Sears
December 1-4, 2014
Orlando, FL
Call for 2013 Cozzarelli Prize Nominations

The PNAS Editorial Board is now accepting nominations through January 10, 2014 for the 2013 Cozzarelli Prize. This award recognizes scientific excellence and will be given to six papers published in PNAS during 2013.

Nominations should be sent to pnas@nas.edu and should include a citation and brief explanation of the merits of the work. The award recipients will be recognized during the PNAS Editorial Board Meeting and the NAS Annual Meeting Awards Ceremony on April 27, 2014 in Washington, DC.

For more information and a list of previous winners visit www.pnas.org/cozzarelliprize.
The following meetings are scheduled to take place in Hong Kong in 2014:

**NEW! Advanced Materials for Sustainable Infrastructure Development**  
*Advanced Materials for Sustainable Energy Efficient Buildings*  
August 3-8, 2014  
The Hong Kong University of Science and Technology  
Chair: Zongjin Li

**NEW! Complex Adaptive Matter**  
*Towards a Unifying Perspective of Emergent Complexity*  
July 13-18, 2014  
The Chinese University of Hong Kong  
Chair: Robert H. Austin

**NEW! Genomic Instability**  
*Mechanisms that Cause DNA Damage and Related Diseases*  
July 6-11, 2014  
The Hong Kong University of Science and Technology  
Chairs: Bik K. Tye & Marco Foiani

**Green Chemistry**  
*Industrial Successes and Challenges*  
July 27 - August 1, 2014  
The Chinese University of Hong Kong  
Chairs: Kenneth Seddon & Mark A. Harmer

**Green Chemistry (GRS)** * Applications for a Sustainable Future  
July 26-27, 2014  
The Chinese University of Hong Kong  
Chair: Magdalena B. Foreiter

**NEW! Hybrid Electronic & Photonic Materials and Phenomena**  
June 22-27, 2014  
The Hong Kong University of Science and Technology  
Chairs: Michael Graetzel & Ben Zhong Tang

**Molecular & Cellular Neurobiology**  
*Mechanisms of Neural Development, Circuit Assembly, Synaptic Plasticity and Neuropsychiatric Disorders*  
June 29 - July 4, 2014  
The Hong Kong University of Science and Technology  
Chair: Eric J. Huang

**Molecular & Cellular Neurobiology (GRS)** * Exploring the Frontiers of Foundational and Translational Neuroscience*  
June 28-29, 2014  
The Hong Kong University of Science and Technology  
Chair: Sarah X. Luo

**NEW! Structural Nanomaterials**  
*Recent Advances in Understanding the Structures and Properties of Nanomaterials*  
July 20-25, 2014  
The Chinese University of Hong Kong  
Chairs: Chain T. Liu & T.G. Nieh

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Living Large: Scaling Up Cell Culture

Whether for structural biology, regenerative medicine, or translational research, basic scientists often find themselves needing larger quantities of cellular products than typical lab-scale techniques can produce. New technologies such as disposable bioreactors and multilayered culture flasks can help address some of the challenges of scaling up. By Alan Dove

“...you can try different types of shaker flasks, different types of spinner flasks that can handle enough volume for initial work, and if you need a little bit more volume you can go into small bioreactors.”

Traditional laboratory biology is a small-scale operation; tissue culture dishes are seldom much larger than the experimenter’s hand, volume measurements are in milliliters, and a protein purification that yields a few micrograms is a success. With burgeoning interest in translational research, structural biology, and regenerative medicine, many scientists are starting to think bigger. Whether trying to purify grams of protein for crystallography or testing the feasibility of turning a novel gene product into a new drug, these researchers soon find themselves pondering the complications of larger-scale cell culture.

Thanks to the success of the biotechnology industry, cell culture scale up has become a well-paved path. “The field has exploded, because a lot of products are being made, drugs are being made in mammalian cells in large quantities in very big bioreactors,” says Joseph Shiloach, head of the Biotechnology Core Lab at the National Institute of Diabetes and Digestive and Kidney Diseases in Bethesda, Maryland. He adds that depending on the cell type, the necessary equipment and reagents are often available off the shelf.

CHECK THE SUSPENSION

For many scientists, the most obvious choice for growing larger batches of cells is a technique they already understand: suspension culture. Numerous cell lines are already adapted to grow in small spinner bottles, so initial scale up can be as simple as buying a larger spinner. Suspension cultures also give researchers multiple options for expanding further.

Cultures in shaker flasks are particularly flexible, allowing researchers to step up through multiple sizes on a single platform—literally. “You can get a small size platform [shaker] that you can put right into your incubator, and … start with tubes that contain just a few milliliters of culture, all the way up to multiple liters of culture in large flasks,” says Henry Chiou, senior product manager for cell biology at Life Technologies in Carlsbad, California.

Suspension cultures adapted to either shakers or spinner bottles are usually easy to expand to even larger sizes if necessary. You can try different types of shaker flasks, different types of spinner flasks that can handle enough volume for initial work, and if you need a little bit more volume you can go into small bioreactors,” says Shiloach.

The biopharmaceutical industry routinely uses suspension cell cultures in bioreactors to manufacture clinically approved products, so vendors produce such systems in sizes that range from benchtop models all the way up to industrial units. Many of these bioreactors now use disposable growth chambers to eliminate the difficulties of sterilization. “The whole bioreactor is disposable, so you can get it already equipped with everything you need, you just have to put in the media,” says continued>
RATHER SWITCH THAN FIGHT

Researchers whose chosen cells don’t grow in suspension need to find another approach to scale up—or not. In many cases, the most sensible choice is to switch to suspension culture. “If you have a product that you want to test and you need a few milligrams just to find out if the biological activity is what you want, you can start doing it in adherent [cells], but if the product seems to be promising, I think it would be better to try and adapt the cells or maybe try another cell line … and then go into suspension,” says Shiloach.

If the goal is a clinical therapy, switching cell lines could also help speed regulatory approval. Established suspension culture systems such as Chinese hamster ovary (CHO) and human embryonic kidney 293 (HEK-293) cells form the backbone of current biopharmaceutical production, so regulators are already comfortable with them.

Switching to a suspension-adapted line is an especially good strategy for scaling up protein production. “When we’ve gone out and talked to lots of folks, there [are] not that many instances where they need to actually be in a specific cell line for protein expression. The standard ones that are typically out there, 293s and CHO kids, seem to fit the vast majority of needs,” says Chiu.

To cater to those needs, several companies offer strains of 293 and CHO cells especially adapted for large-scale suspension growth and protein production. For example, Life Technologies’ Exp293 system uses a special strain of 293 cells and a proprietary medium to achieve high levels of transient protein expression. The company claims Exp293 can produce three- to fivefold more protein per liter than conventional culture systems. Higher production means less need for larger-scale cultures. “In the past if somebody felt like they needed to scale up to a 5 L reaction in order to get enough protein, they can now stick with a 1 L or 2 L reaction,” says Chiu, adding that “that really helps to lower the impact of scaling that they need to do.”

Even increasing the required culture size a little bit can often entail major increases in cost and complexity, as scaling problems tend to be nonlinear. Moving from 1 L of culture to 2 L might only entail using a bigger shaker flask, but moving from 5 L to 6 L could mean buying a benchtop bioreactor and learning how to use it. “The inflection points tend to happen as you change the type of vessel you’re using,” says Chiu.

When standard protein expression lines such as CHO and 293 won’t work—perhaps because of a need for specific glycosylation patterns that these cells can’t perform—some researchers turn to microcarriers. These tiny plastic beads provide a surface for adherent cells to bind. Once seeded, the microcarriers and their attached cells can be suspended in shaker or spinner flasks. This decades-old technique provides many of the benefits of suspension culture for cells that can’t adapt to it otherwise. Microcarriers are best suited for protein or virus production, as recovering viable cells from the beads can be tricky. Several equipment suppliers offer microcarriers, including GE and Sigma-Aldrich as well as Labtech of Uckfield, United Kingdom.

MORE IS MORE

For some cell types and projects, none of the existing suspension culture
systems will work. Stem cells are particularly reluctant to grow in suspension, and Vero cells, a common line for vaccine production, also need to attach firmly to plates. Fortunately, scientists trying to grow larger batches of such adamantly adherent cells can choose from several well-developed techniques.

The simplest strategy is to increase the available surface area in each culture flask, by using special flasks with multiple layers of plates inside. This parallel plate approach is somewhat more cumbersome than suspension cultures, but equipment manufacturers have worked hard to optimize it. “We were the first company that came up with this design decades ago with the Cell Factory, and we have a variety of choices in that particular product line,” says Cindy Neeley, field applications specialist for cell culture at Thermo Fisher in Waltham, Massachusetts. Other companies also offer layered bioreactors for adherent cells, often optimized for specific uses. For example, the CELLine bioreactors from Sigma-Aldrich in St. Louis, Missouri are specifically designed for scaling up protein expression systems and monoclonal antibody production.

Moving cells from small single-layer culture flasks into parallel plate vessels brings its own set of challenges. Neeley points out that even though all of the plates in a multilayer flask are right next to each other, conditions can vary from one layer to another. “Anything more than 10 layers, you’re going to encounter issues with distribution of nutrients as well as gas conditions between layers, and that is obviously a challenge for us,” says Neeley. As a result, large parallel plate systems often come with special support equipment.

When shopping for such a system, researchers should also consider what surface their cells currently prefer. Cells adapted to adhere to one type of coated flask may grow poorly when moved to a multilayer flask with a different coating. For established cell lines, the solution is often as easy as buying a parallel plate system from the same vendor that provided the smaller flasks, to ensure the coatings are the same.

That won’t work for researchers at the cutting edge of regenerative medicine, though. Stem cells are often too finicky to grow on any of the generic surfaces available off the shelf, instead requiring special coating reagents such as BD Biosciences’ Matrigel. Coating a large parallel plate flask with such reagents would be cumbersome and costly. That puts parallel plate systems out of reach for stem cell work, at least for now. “We’re not there yet, but companies in our position need to think in that direction, to come up with … vessels that would allow these stem cells to attach and grow,” says Neeley.

For cells that can grow in parallel plate flasks, though, subsequent increases in scale—all the way up to industrial sizes—are relatively straightforward. Indeed, the largest version of Thermo’s Cell Factory is already a common sight in vaccine production facilities, where the flasks are so huge that simply moving them around is one of the biggest challenges.

The difficulty of manipulating multiple large parallel plate flasks has led other equipment developers to create more compact systems. The xPansion multilayer bioreactor from ATMI in Brussels, Belgium uses thinner plates and puts them closer together than the Thermo Cell Factory. That allows the xPansion to pack 20 Cell Factories’ worth of cells into a single container. Besides saving space, the more compact system reduces the number of flasks that have to be manipulated each time technicians need to change media or harvest cells.

Like other flask makers, ATMI is also trying to coat its plates with surfaces suitable for more exotic cell types. “Today we have only one coating, but we are actually working on specific coatings [for stem cells],” says Jose Castillo, director of cell culture technologies at ATMI.

**GOING WITH THE FLOW**

Researchers who are using adherent cells as expression systems for secreted proteins should also consider another strategy for scaling up: fixed-bed bioreactors. In this approach, cells adhere to small beads or fibers that are packed into a large container. Medium circulates through the matrix continuously, bringing fresh nutrients and oxygen into the bed and pulling cellular waste and secreted proteins out. It’s difficult or impossible to recover the cells from the fixed bed once they’ve adhered, but for secreted protein production that isn’t usually a problem.

Fixed-bed systems can fit a large number of adherent cells into a relatively small volume. The iCells 500, for example, holds 500 m³ of adherent cell culture surface—roughly two tennis courts’ worth—in a volume of only 25 L. The high surface-to-volume ratio can make fixed-bed systems even better than suspension cultures for protein expression and viral vaccine production. “You will be able to optimize your perfusion process in such a way that the [product] is actually harvested into a smaller volume when compared to the reference process of a stirred tank bioreactor,” says Castillo. This makes the iCells 500 appropriate for laboratory as well as industry applications.

Whatever types of cell cultures researchers need to expand, the recent technological advances by equipment makers have smoothed the path considerably. Shiloach explains that just in the past few years, “the media are much better, the cell lines are better, also they have better ways of doing all the molecular biology. It seems to me that it’s much more difficult to identify the right product than to produce it.”

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Alan Dove is a science writer and editor based in Massachusetts.

DOI: 10.1126/science.opms.p1300080
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Abstract Deadline: December 18, 2013
Discounted Registration Deadline: January 21, 2014

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CONFIRMED SPEAKERS (as of November 4, 2013):
Laura Clarke, European Bioinformatics Institute
Mark Gerstein, Yale University
David Haussler*, UC Santa Cruz
Jill P. Mesirov, Broad Institute
John Overington, European Molecular Biology Laboratory
Ajay Royyuru, IBM T.J. Watson Research Center
Michael Schatz, Cold Spring Harbor Laboratory
Dan Stanzione, University of Texas at Austin
Lincoln D. Stein, Ontario Institute for Cancer Research
Susan Sunkin, Allen Institute for Brain Science
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