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Confluent Trends in Cell Culture Media

Animal-Product-Free and Chemically Defined Media Are Enabling Diverse Applications

- *Angelo DePalma, Ph.D.*

The dramatic rise in titers of recombinant therapeutics, particularly antibodies, is due in large part (some would say entirely) to improved culture medium and feed strategies. Improved media have additionally fueled the emergence of three-dimensional cell culture, stem cell culture, cell-based assays, and the resurgence of primary cell culture.

As a result, cell culture media is a growing, thriving business. According to a new market report published by Transparency Market Research, the global market for cell culture media, sera, and reagents market will grow to $7.1 billion by 2023. This report, which is entitled “Cell Culture Media, Sera, and Reagents Market—Global Industry Analysis, Size, Share, Growth, Trends, and Forecast 2015–2023,” notes that since the market was valued at $3.7 billion in 2014, the estimated value for 2013 reflects a brisk growth rate of 7.6%.

Over the past decade or so, various cell culture media trends have emerged. These include the elimination of animal-origin components and the ability to support high titers. Going forward, these trends may coalesce, giving bioprocessors the chance to explore how media development can relate to larger issues such as workflow efficiency and quality assurance.

Media/Workflow Dynamics

“People have achieved the defined formulations and titers that meet their expectations, and these capabilities are permeating throughout bioprocessing today,” says Brandon Pence, global marketing leader, bioprocess, GE Healthcare. “Now the emphasis is on characterizing the medium’s influence on protein structure and quality.”

To assess critical quality attributes, bioprocessors leverage their understanding of cell culture systems. In addition, they wield various analytical tools to see how cell culture conditions and quality attributes relate to each other. Exploring these connections has become a leading issue for bioprocessors, particularly for those involved in the development of biosimilars.

Media development also plays into a second trend, namely, designing efficient bioprocessing workflows. “How do we take the molecule expressed upstream and move it to a downstream system designed for it?” Pence asks. He notes that a “holistic view” of the bioprocessing workflow will improve understanding of how process environments affect expressed proteins, and what that means from a downstream perspective.

“There’s a buzz around continuous processing,” Pence explains. “Whether you deploy it or not, the idea of linking upstream to downstream operations is important, as upstream conditions influence downstream purification. GE spends considerable resources on understanding the end-to-end bioprocessing workflow.”

For example, certain culture conditions may prevent or promote cell clumping or shear damage, while others may lead to incorrect protein structure. “It’s not uncommon to solve an upstream issue and thereby create a downstream problem,” Pence observes. “But when adjustments are made with the complete system in mind, these situations can be largely avoided.”

Optimizing Base Ingredients

The advantages of highly concentrated feeds and media have been known for some time. Concentration allows for higher cell density, improved economics in the form of lower volumes of media in storage, lower feed rates, and higher volumetric productivity.

Concentrated media may be prepared more easily through the use of technology developed by MilliporeSigma, a U.S. life science business of Darmstadt, Germany-based Merck KGaA. The business emerged from Merck KGaA’s recently completed acquisition of Sigma-Aldrich.

One technology that is available through the new business involves enhancing the solubility of the final media product. In June 2015, the parent company of the new business introduced a solids compaction technology that speeds dissolution of highly concentrated media. The technique compacts dry powder media into granules, thereby accelerating solubility, improving dry media flow and handling, and cutting down on dust.

A second technology addresses poorly soluble critical ingredients such as ferric citrate, a common iron source for chemically defined media that lack transferrin. The solubility of ferric citrate is just 0.002 g/L at room temperature and neutral pH. “If you cook it, you can get 5 g/L,” notes Nikolai Stankiewicz, Ph.D., head of MilliporeSigma’s technology transfer laboratory. “But that’s not feasible with cell cultures.” Through a special freeze-drying process, 2g/L concentrations are possible.

A third technology involves pluronic, a nonionic copolymer surfactant that protects cells from shear force damage, thus enabling higher cell densities. “Pluronic is used in virtually every mammalian cell culture,” Dr. Stankiewicz explains. “Lot-to-lot variability of this chemical is a significant issue. Until recently, there was no method for evaluating the quality of pluoronic batches at small scale.”

Several bioprocessors have traced poor or variable culture performance back to pluronic quality. MilliporeSigma has developed a physical-chemical assay for pluronic. The assay, which is based on analytical chromatography, is used by the company to test all the pluronic batches that it uses for cell culture media production.

Physiologic Relevance

Interest in primary cell culture is causing a shift in cell culture media preferences, according to Nicol Watson, Ph.D., senior global marketing manager at Thermo Fisher Scientific, which surveys customers annually about such things.

“A good indicator,” says Dr. Watson, “is that customers are purchasing more chemically defined media as well as newly introduced transfection reagents that are more efficient for difficult-to-transfect cells, but also gentler on them.”

Interest in primary cells has caused an uptick in Thermo Fisher’s custom media business. “Customers are looking for a slightly leaner medium, to which they add various growth factors and substrates,” Dr. Watson adds. “There’s definitely more cooking involved in looking after primary cells.”

Some primary cell work relies on cells harvested from patients and cadavers. Induced pluripotent stem cells (iPSCs), with their own special media requirements, are the other main source of primary cells for achieving physiologically relevant research models.

Harvesting would seem to provide limitless numbers of primary cells, but the process is mostly manual. Moreover, the cells last through just five or six passages. Many somatic cells cannot be propagated at all, so their supplies are limited extremely. “You simply can’t produce as many cells through harvesting as you can by turning somatic cells to iPSCs followed by differentiation,” Dr. Watson tells GEN.

Customers continue to deal with serum shortages and skyrocketing prices by employing reduced-serum and serum-free media whenever possible. Dr. Watson observes that this trend holds for both academia and industry, and wherever cells have any potential for eventual in vivo therapeutic use.

According to Dr. Watson, his customers are showing increasing interest in less complex media. “Complexity” in media refers to the number of components. “Less complex media often contains more complex ingredients,” clarifies Dr. Watson, “but fewer of them.”

“Instead of specifying DMEM media, which contains 47 components, certain customers prefer better-defined media with even fewer components,” he continues.

Dr. Watson points out that a parallel trend is occurring in stem cell culture, where feeder-free systems are replacing feeder-based cultures: “Now they’re moving toward more defined and specialty media where the feeding regime becomes less burdensome.”

On the subject of serum-free media for cells with therapeutic potential, Celprogen has recently introduced a novel serum substitute, XFS2, specifically tailored to the needs of human cell culture. XFS2 supports the culture of stem cells, progenitor cells, induced pluripotent cells, and primary human cells. XFS2 is also suitable for culturing cancer stem cells, circulating tumor cells, and differentiated parental tumor cells.

Interestingly, the secret behind XFS2 is not an exotic cellular or plant extract. Instead, XFS2 relies on synthetic ingredients that apparently do the job of animal sera. That is, XFS2 allows for chemically defined media for human cell culture.

"There is significant variability with fetal bovine serum," explains Jay Sharma, Ph.D., Celprogen's CEO. "XFS2 is the same lot after lot, which provides consistency of results."

By using a serum substitute, scientists can avoid the need to wean cells from a serum-based medium to serum-free conditions, thus avoiding the well-known technical and regulatory issues related to animal-component-containing products. The serum to no-serum switch takes about one week, according to Dr. Sharma. In addition to eliminating the delay associated with this switch, a serum substitute can ensure that cells will have the same characteristics throughout the research timeline.

In November 2015, Takara Bio Europe launched its hepatocyte differentiation system, a medium kit for differentiating iPSCs to hepatocytes. These hepatocytes have high expression levels of drug-metabolizing enzymes and transporters, and retain functionality for at least 11 days. Takara Bio has been working with stem cells since the early 2000s, and has developed protocols for generating hepatocytes from 25 different iPSC lines.

"Culturing pluripotent stem cells in a robust manner, one that maintains pluripotency and prevents differentiation, and that ultimately allows reproducible differentiation, is a clear challenge," says Kristina Runeberg, site head and senior director for business development at Takara Bio. "It requires an advanced culture media system to ensure that the differentiation protocol begins with a homogenous population of undifferentiated single cells. Having reliable starting materials facilitates reproducible differentiation into the cell type of interest."

Currently, most groups are culturing pluripotent cells in flasks. To lower costs of labor, culture media, and reagents, Takara Bio cultures iPSCs in a 3D environment whenever possible. "We are developing a culture system for suspension culture of pluripotent cells and downstream differentiation," says Runeberg. "In addition to a cost savings benefit, 3D culture better represents the way cells grow in vivo."

Because therapeutic doses of stem cells require as many as 1010 cells, cultures (including media) must be tightly quality-controlled and begin with large numbers of undifferentiated cells. Takara Bio has developed a GMP-grade version of its DEF-CS culture system that is free of animal-derived components, is chemically defined, and contains material of clinical quality with traceable production processes.

Quality's the Thing

Bruce Lehr, senior manager of R&D at MilliporeSigma, notes a significant resurgence in perfusion cell culture, in part enabled by single-use equipment that allows operation at very high cell densities. Media for dense perfusion cultures

must support cell densities of up to 150 million/mL, compared with 20 million/mL for fed-batch cultures. Concomitant reductions in bioreactor volumes, Lehr explains, have improved process economics: “Capital costs are much lower for facilities that rely on single use, and plants can be more flexible.”

Media must support these higher densities by providing superior nutrition and waste product management while limiting media changes. “Processors are working toward one reactor volume change per day, versus the five or more that were common 20 years ago,” Lehr adds.

MilliporeSigma has been working on proprietary perfusion media formulations for customers, and it expects eventually to offer similar catalog products.

Glycosylation is a somewhat controversial topic, particularly for follow-on biologics, but the general consensus has been that biosimilars resembling originator molecules in glycosylation patterns have a greater likelihood to succeed. One way to control glycosylation is through formulation or via supplementation. MilliporeSigma’s Ex-Cell® Glycosylation Adjust (Gal+), for example, allows a transition from less mature (less glycosylated) to more mature (more glycosylated) forms of target proteins, which allows processors to fine-tune and replicate as closely as possible glycosylation of the originator molecule. “Ultimately,” notes Lehr, “you have to match whatever the licensed product has, good or bad.”

As part of the general focus on quality, media vendors are looking more closely than ever at components and raw ingredients, which also affect glycosylation. Mined minerals, in particular, can carry over trace elements that vary from batch to batch and often go undetected. MilliporeSigma has therefore begun characterizing all its raw media ingredients with the goal of improved control over what Lehr refers to as the “finer aspects of manufacturing,” such as glycosylation. “Only in last few years,” observes Lehr, “have companies had the luxury (and tools) to systematically look for these things.”