Pharmaceutical BIOPROCESSING

Development of protein-free medium for therapeutic protein production in mammalian cells: recent advances and perspectives

Mammalian cell culture media used for the manufacture of therapeutic protein have advanced systematically from serum-containing into animal-free, protein-free and chemically defined formulations over the past decades. Initially driven by patient safety concerns associated with the use of animal-derived medium components, and later by inconsistent cell culture performance due to variability in plant-derived raw material lots, many biologics manufacturers redirect their focus on the development of proprietary media formulations and implementation of well-controlled chemically defined raw materials in all cell culture media and feeds for production processes. This article will provide an overview of current trends and objectives of industrial medium development efforts for therapeutic protein production.

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Global market shares of protein biologics have continued to rise despite small molecule therapeutics dominance. Forecast for the global biologics market has been projected to reach over US$200 billion by 2016 and $250 billion by 2020 [1,2] out of more than $1 trillion of total global pharmaceutical market [3].

Cell lines

More than 90 biologics have been approved by the US FDA as of September 2014 [4]. The cell expression systems responsible for current approved biologics include mammalian, microbial and yeast cells. Greater than 50% of these biologic products are expressed in mammalian cells, with Chinese hamster ovary (CHO) cells as the predominated expression system [5,6]. Two CHO expression systems commonly used in biologics manufacture utilize the dihydrofolate reductase (DHFR) and the glutamine synthetase (GS) selection principles. Both expression systems have resulted in multiple grams per liter productivity in 10–21 day fed-batch processes [7–13]. The history of their development has been described in detail by Wurm [14] (Figure 1). CHO-K1 and CHO-DG44 cell lines were resulted from further subcloning from their progenitor CHO-ori cell line. CHO-K1 cells were subsequently used to generate the CHO-DXB11 and CHOK1 SV cell lines. The CHO-DXB11 host cell system has a deleted DHFR locus and a mutated second DHFR locus. The CHO-DG44 host cell system has both DHFR loci deleted. When the gene of interest (GOI) is transfected with the *DHFR* gene into DHFR deficient cells, cells that can successfully express the GOI are those that demonstrate methotrexate resistance [15–17]. The CHO-K1 SV host system expressing low level of GS is termed CHO-GS (or GS-CHO) cell line. In the CHO-GS system, untransfected CHO cells having low level of endogenous GS are unable to grow in the presence of methionine sulfoximine, a GS inhibitor and glutamate analogue. When these CHO host cells are transfected with a vector carrying *GS* gene with the GOI, the cells (CHO-K1 SV) are able to synthesize glutamine from glutamate in the presence of methionine sulfoximine and express the protein of interest. The objective of cell line development is to ensure the production cell line stability through the end of production cell age. When therapeutic protein expressing cell lines are selected, preparation and testing of the manufacturing cell banks [18–21] are necessary to ensure the

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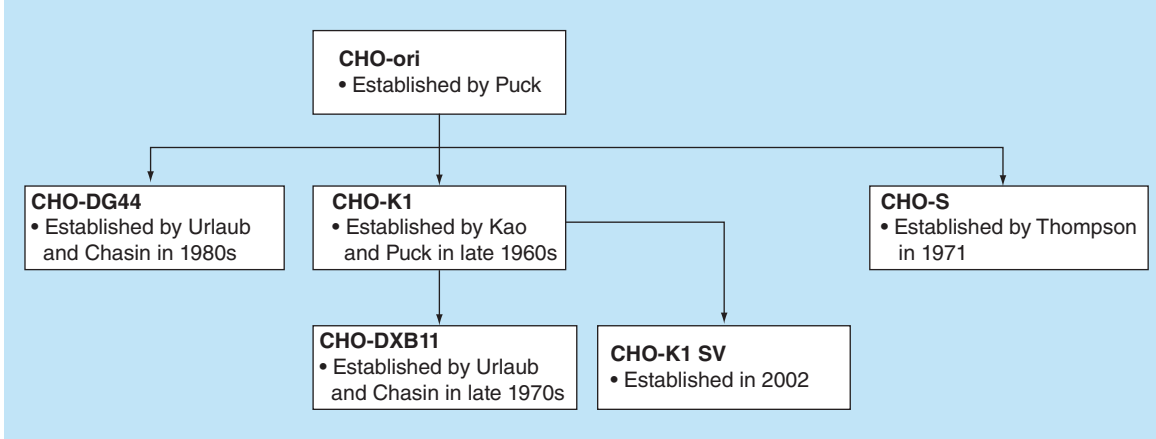

Figure 1. Development history of Chinese hamster ovary cell lines.
CHO: Chinese hamster ovary.

production host cell lines are free of adventitious agents prior to GMP manufacturing. The cell culture media and feeds that are used to propagate the cells and produce the final therapeutic protein products must also meet these testing requirements.

Cell culture medium development history

Cell culture media provide nutrients for the cells to propagate and produce proteins. Typical cell culture media contain 50–100 components that can be grouped as trace elements, inorganic salts, energy source, amino acids, vitamins, nucleic acid derivatives, fatty acids and lipids, and others [22,23]. Due to their ease of use, complex medium components such as serum which served as cellular protectants against shear stress have been favored by the development groups in the past. In the 1950–1960s, polio vaccines produced in adherent monkey kidney cells were manufactured with serum-containing media. CHO and mouse myeloma NS0 processes included bovine serum to protect cultures from shear, pH and nutrient stress in bioreactors. However, concerns of undefined raw material potentially associated with presence of adventitious agents, bacterial endotoxin, immunogenic contaminants, fungi, prions, bacteria and mycoplasmas began to surface and have been acknowledged by the regulators and drug manufacturers. The subsequent mycoplasma scare in the 1970s, endotoxin incidence in the 1980s and bovine spongiform encephalopathy/transmissible spongiform encephalopathy (BSE/TSE) concerns in the 1990s have placed a priority to the regulators and the drug manufacturers to actively remedy these safety issues with the replacement of defined raw material in the manufacturing processes. In the 1950–1960s, viral contamination in polio vaccine was identified in the SV40 contaminated cell source. This finding resulted in the establishment of production standards for drugs for human use. In the 1990s, Genentech's parvovirus Minute virus of mice contamination which affected CHO-based processes, led to the use of parvovirus, a nonenveloped single-stranded rodent virus, as the current standard model virus to demonstrate viral removal and inactivation. Vesivirus contamination in 2009 at Genzyme resulted in CHO cell growth inhibition [24]. The viral contamination subsequently was traced to a nutrient additive. This incident led to the development of PCR-based detection methods for vesivirus to support in-process sample analysis and viral clearance studies. These experiences and concerns are responsible for the continued refinement of the ICH guidelines on quality (Table 1).

The initial concern on raw material was primarily replacing animal-derived components with nonanimal derived but complex counterparts, such as nonanimal hydrolysates. Common marketed protein hydrolysates are produced from soybean, rice, wheat, pea, cotton and yeast. The hydrolysates, which are low cost substitute to serum, serve as cellular protectants

Key terms

Chinese hamster ovary cells: Most common mammalian expression system used in the manufacture of protein therapeutics. The two most prevalent Chinese hamster ovary systems utilize the dihydrofolate reductase and the glutamine synthetase selection principles for protein production. Both expression systems have resulted in multiple grams per liter productivity in 10–21 day fed-batch processes.

Adventitious agents: Contaminants that pose safety concerns in the therapeutic products. In mammalian cell culture, adventitious agents include viruses, TSE/BSE, DNA, mycoplasma, endotoxin and bacteria. Adventitious agents may come from medium component source, cell lines, manufacture facility and any steps in manufacture. Drug manufacturers must demonstrate their products are free of adventitious agents prior to human use.

Table 1. Safety concerns that changed manufacture practices.

Time	Concern	Mitigation	Guidance
1950s–1960s	Virus contaminant from monkey cells used in production of polio vaccine	• Consistent GMP manufacturing • Demonstrate drug effectiveness and safety	• Division of Biologics Standards created in 1955 within NIH • Kefauver-Harris Drug Amendments in 1962 to Food, Drug and Cosmetic Act of 1938
1970s	Mycoplasma contamination due to sera products	• Raw material, cell bank testing	• Bureau of Biologics transferred from NIH to FDA
1980s	Endotoxin contamination in cell culture	• Raw material control (sera, media components, water, labware) • Endotoxin testing limit setting	• USP <85> Bacterial endotoxins test: – 1980: USP XX. pp. 888-889 – 1984: USP XXI. pp. 1165-1167 – 1989: USP XXII. pp. 1493-1495 • FDA 1987: Guidance on validation of the Limulus Amebocyte Lysate test as an end product endotoxin test for human and animal parenteral drugs, biological products and medical devices
1990s	Potential contamination of bovine-derived materials linked to BSE/TSE	• Stringent requirement for serum and raw material from animal-derived sources	• 1993: Points to consider in the characterization of cell lines used for the production of biologics
2000s	Viral contamination	• Use of raw material free of animal-derived sources and protein-free • Raw material testing	• 1996: ICH Q5A: quality of biotechnology products: viral safety evaluation of biotechnology products derived from cell lines of human or animal origin • 1997: Points to consider in the manufacture and testing of monoclonal antibody products for human use

BSE: Bovine spongiform encephalopathy; ICH: International conference on harmonisation; TSE: Transmissible spongiform encephalopathy; USP: US pharmacopeia. Data taken from [25–30].

against shear stress and enhance cell mass and viability (Table 2). However, as production demand increases, lot-to-lot variations in hydrolysates not only have impacted product purification and yield, but also have hampered cell culture production consistency. This, in turn, triggers strategic work in blending different hydrolysate lots to ensure comparable culture batch performance.

Cell culture media for mammalian cells also have progressed from formulations containing complex, animal-derived components to those of protein-free and chemically defined nature. The current preferred medium formulations for drug manufacture are those with completely chemically defined components. This strategy minimizes potential safety concerns associated with animal-derived raw material and reduces raw material lot-to-lot inconsistency. Moreover, drug manufacturers have insisted on higher degrees of animal-free origin raw material for all medium components. Those with primary degree of animal-free origin designation are expected as they are in contact with therapeutic products. Some medium vendors have implemented raw material qualification sourcing strategy to identify and source raw material with secondary and tertiary degrees of animal-free origin. Such extensive efforts are pursued to ensure the final therapeutic products are free from animal-derived components throughout the history of the raw material origin and manufacture.

As a part of life cycle management, raw material in legacy media that contain serum and undefined components are being phased out to support today's standards. To meet drug demand as well as ensure patient safety and regulatory acceptance, raw material

Table 2. Considerations in cell culture medium development.

Considerations	Complex components	Chemically defined components
Advantages	• Cost effective • Promote high cell mass and viability • Enhance production titers • Cellular protectant	• Elemental composition known • Facilitate medium development and optimization • Opportunity to identify specific raw material sourcing suppliers • Meet regulatory guidelines
Disadvantages	• High raw material variability • May be animal derived • Potential presence of contaminants • Potential process and product inconsistencies • Potential presence of growth inhibitors • Impede medium development and optimization	• Can be expensive and may have limited sourcing suppliers • May not have cellular protectant properties as complex components • Reduced growth rate or viability • Potential decreased titers

and production processes are valued as critical components as much as biologic productivity. Assessment plans are staged to compare and bridge products manufactured with new and previous raw material in preclinical and clinical settings.

General medium development strategies

In addition to mitigate potential introduction of adventitious agents through the use of undefined raw material, developing chemically defined media provides opportunities to establish a common set or 'platform' media that target selected expression host systems and specific product quality profiles, and meet production operations setup.

Medium design

Most therapeutic proteins especially monoclonal antibodies (mAb) are produced from CHO cells transfected with the genes of interest. Minor cell handling variations over the years for the same CHO strain in different laboratories have rendered some differences observed from the original CHO host. Therefore, it is not unusual that specific preferences in growth conditions or performance are observed for the cell lines by different laboratories. The typical medium design starts with a medium of known compositions. Early chemically defined media are formulated with amino acids, vitamins, antioxidants, trace metals, lipids, inorganic buffers and salts and others. The impact of the new media was evaluated for their cell culture performance in a number of cell lines. Initial medium formulations are typically liquids derived from mixtures of component groups defined from historical experience and literature review. Multiple medium compositions can be formulated to establish a medium library for component screening of specific performance profiles for cell lines of interest. The library can be further expanded with additional cell lines evaluated. Spent medium analyses are conducted to refine the media through multiple rounds of optimization. When a number of internal mAb-expressing cell lines are evaluated under a fixed procedure, a common set of media and feed can be established. This common medium set is termed the 'platform media' and the fixed procedure becomes the 'platform process' (Figure 2). The platform system allows development organization to move biologic programs that utilize the same CHO host system and platform production process through development into the clinic rapidly. Additional refinement to media and feed concentration can be optimized at a later stage.

The development focus is to ensure nutrient balance to improve growth rates and maintain high cell viability. The growth media contain 50–100 components where the concentrations have been optimized and balanced with feed media for production. Traditionally, the compositions are optimized one factor at a time which can be labor-intensive and time-consuming. Today, statistical designs have dominated medium screening strategies to reduce workload and enhance statistical power and speed to formulation identification. Plackett–Burman [31,32], factorial [33,34], central composite and response surface [35] designs coupled with multivariate analysis [36] have supported development of successful chemically defined formulations.

In addition to support high productivity, the technical focus of these chemically defined media includes: ensuring genomic stability and viability [37], confirming proper cell cycle to progression [38,39], reducing potential

apoptotic pathway activation [40–43], minimizing production of toxic metabolites [44–46], maintaining efficient metabolic enzyme activities [47–49] and minimizing cell stress [50–53]. Consideration of 'nutrient shift' during cell growth and protein production indicates a modification of components in the media and feed composition as the production batch progresses. The nutrient shift is designed to convert cultures from one metabolic state to another. For example, cultures in lactate producing state can be shifted to lactate consuming state by changing pH control parameters or modifying feed rates. Cell cycle G1/S phase arrest has been a target for production medium and feed development [54,55]. Fed-batch cultures in exponential growth phase can be converted to stationary phase with appropriate feed supplementation and process conditions. The intention of feed development is to enhance specific productivity with reduced focus on significant nutrient balance. Other supplementation includes the glutamine-based peptides [46,56], or tricarboxylic acid cycle intermediates [49,57] to reduce rate of inhibitory metabolite formulation. Ideal growth media ultimately allow continued cell expansion from vial thaw to support expansion of highly viable inoculum, and production media and feeds focus on enhancing specific productivity.

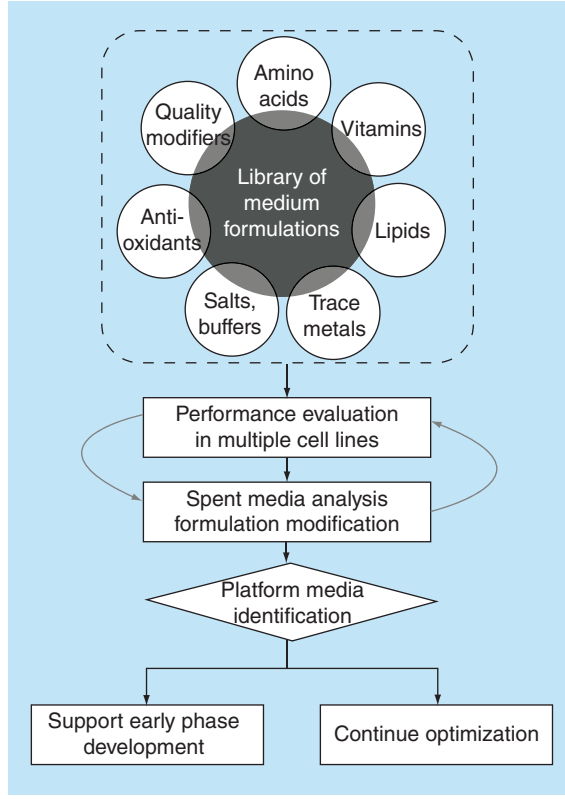

Figure 2. Platform medium development.

Protein quality profiles

Product quality such as degree and profile of charge distribution and glycan moieties of therapeutic proteins can have a significant role in determining efficacy in animal and/or human PK [58–60]. Cell culture media and feeds and process strategy have an important effect on product quality. Copper in cell culture medium has been shown to enhance cell viability and facilitates disulfide bond formation [61]. Sodium butyrate, an inhibitor of histone deacetylase activity that mediates cell proliferation arrest [62], has been demonstrated to enhance gene accessibility leading to observed increase in specific productivity [63]. In addition, sodium butyrate has been shown to improve mAb assembly [64] and to reduce protein sialylation [65,66]. Undefined component such as serum in media supplemented in CHO cells produced proteins with higher level of terminal galactosylation than serum-free media [67,68]. Amino acid supplementation typically used to enhance cell growth and productivity has been shown to reduce sialylation [69]. Supplementation of *N*-acetylmannosamine to CHO cells increases the intracellular precursor pool of CMP-sialic acid [70]. Glycosylation predictive strategies employing visualization tool to monitor glycan distribution due to culture condition changes [71], mathematical model linking cell growth and metabolism to glycosylation [72] and statistically designs for on-line glycosylation control [73] can be utilized to support media development for both novel biologics and biosimilars.

Medium manufacture considerations

Medium development scientists tend to work with concentrated liquid stocks where components of similar properties are grouped together in stock solutions. These stocks easily facilitate selected mixing and blending to generate new formulations. While blending of formulations is common at lab scale, it is operationally challenging at large-scale. Therefore, liquid media are usually implemented early in development where concentrated stock solutions can be combined to generate different formulations. Liquid media tend to have shorter shelf life than powder media, and large volume

Key terms

Chemically defined formulations: All components in medium formulations are chemically synthesized or purified to homogeneity, and free of animal-derived material.

Protein production: Process by which specific proteins are produced in Chinese hamster ovary or mammalian cells under control conditions. The controlled production processes require cell culture media and feed supplements to propagate the cells and trigger the release of the proteins of interest.

of liquid are more difficult to be shipped. When media become finalized, formulations in powder formats in general are preferred at large-scale. The preference of powder media over liquid media is due mainly for their low cost, long shelf lives and ease of transport (Table 3). Although the same media formulation was used to manufacture both liquid or powder formats, the final prepared liquid or powder-hydrated media may be different to the original theoretical component composition and have different cell culture performance. Physiochemical variations in the same formulation exist [74] when the manufacture is not well defined. Differences in color, pH, osmolality, particle size distribution, humidity have been detected from lot-to-lot of powder media and have resulted in variability in cell culture performance. To mitigate these potential issues, many media manufacturers are including control steps to monitor raw material quality to ensure consistency in raw material selected and their suppliers audited, and explore better milling and blending and new powder manufacturing technology. Raw material degradation or stability monitoring has led to the implementation of sophisticated analytical methods to detect amino acid concentrations in the formulation using NMR- or UPLC-based technology and monitor chemical integrity using NIR. These analytical results and profiles obtained from these preventive steps further ensure newly manufactured lots are consistent with those of historical lots. The combination of media storage stability analyses and end-user cell culture testing provide confidence on media integrity prior to their use.

Ball milling technology which has been used to produce powder media, requires extensive cleaning and sanitization and generates debris residues resulted from continued pulverization of medium components with ceramic balls. In addition, this milling process has posted a number of challenges in: ensuring trace component homogeneity in the final formulation, reducing heat generation especially for heat-sensitive components and maintaining reconstitution solubility in powder media after milling. As such the ball milling process is mostly reserved for legacy products and has very limited use for powder media manufacture today. Currently hammer mills or jet mills with micronization eliminate most of these concerns, and are the more prevalent milling devices to generate powder media. Particle size monitoring technologies, such as laser diffraction, have a role in understanding the mixing effects, solubility, oxidation, microbial degradation and air jet sieving. These quality checks together form the 'fingerprint' of the powder formulation. Fluid bed granulation technology has further simplified liquid reconstitution without the need for pH adjustment. However, this technology remains proprietary to specific media vendors.

Medium development driven by therapeutic protein production strategy

Production processes and manufacturing capacity and facilities play an important role in the direction of medium development strategies. In general, the two main cell culture process steps are cell inoculum scale-up and production (Table 4). Growth or base media support inoculum scale-up stage with the goal of generating high quality, viable cells that are necessary for high productivity. Production media and feeds support production stage with the goal of producing high therapeutic protein titer. For most fed-batch processes, the media component typically is consisted of a set of growth and concentrated production feed media for each cell line. For continuous perfusion processes, one medium is usually required. More recently, a mix of these two traditional processes have become more common. One process which includes a combination of a fed-batch inoculum scale-up step and a fed-batch production step utilizes a growth medium and 1–2 production feed

Table 3. Properties of liquid and powder media.

Considerations	Liquid media	Powder media
Stage of implementation	• Starting point of development • Easy to modify formulations with concentrated stock solutions	• After stage-defined formulation identified
Formulation consistency • Component 'fingerprinting' • Cell culture performance	• Shelf life stability sensitive to storage conditions	• Sensitive to manufacture process, storage condition and hydration process
Shelf life	• Typically limited	• Typically long
Shipping and transfer • Temperature excursion • Quantity logistics	• More challenging	• Preferred

Table 4. Roles of media and feeds in different process steps and production strategies.

Process step	Operation	Cell density	Cell quality	Medium/feed demand
Inoculum scale up • High quality cell mass	Standard assage	Low	High	Low
	Fed-batch	Medium	Medium	Low
	Perfusion intensification	High	High	High
Production • High protein productivity	Fed-batch	Medium	Medium	Low/medium
	Perfusion intensification	High	High	High

media. Another process which requires a perfusion step for inoculum scale-up and a fed-batch production step uses a growth medium for perfusion and a production feed media.

Most commercial facilities can support batch and fed-batch processes which require low medium preparation and storage capacities. Concentrated feeds are ideal for fed-batch processes with feed volume constraint. Perfusion processes also have a long history in protein production. These processes are primarily supporting adherent cultures to produce early biologics [75–77] and stability labile proteins and enzymes [78,79]. However, perfusion processes have large medium requirements. Continuous processing strategy where culture perfusate is continuously purified in small batches also necessitates large medium volume preparation and storage. These large medium volume requirements would necessitate medium cost to be low, and liquid media stability to be adaptable to medium preparation schedule. A production strategy that utilizes a combination of perfusion for inoculum scale-up and fed-batch production processes falls into the intermediate medium needs. Strategic focus for medium and feed development is expected to be different for fed-batch, perfusion and the combination of perfusion and fed-batch processes. The compatibility of growth media to feed media supporting the multitude of processes requires substantial stepwise and targeted development strategic focus.

Media for routine inoculum scale-up

In general, inoculum scale-up requires continued propagation and expansion of seed train to a target cell density until the production stage. Typically, target cell inoculation densities of less than 1×10^6 cells/ml and inoculum quality from each scale-up stage is expected to be similar to inocula from previous scale-up steps. As such, robust inoculum expansion is expected to include optimized procedures for vial thaw with high cell viability recovery, seed density having low doubling time to reach higher peak cell densities and culture pH that aligns with CO_2 concentration set-points of the process [80]. Each scale-up step has the duration of 3–4 days. Therefore, growth media only require sufficient nutrient levels to support cell expansion of 3–4 cell doublings of high quality cell inoculum. The focus of medium development for growth media would be to support high specific cell growth rates with high cell viability and minimize lag phase of growth.

Media for fed-batch inoculum preparation

Fed-batch inoculum preparation tends to be targeted at the N-1 and/or N-2 stages with the goal to increase cell density by 1–2 folds that of traditional cell expansion step but in 5–6 days. In order to support N-production strategies that require high inoculation cell densities (>3 $\times 10^6$ cells/ml), the challenge is developing feed media formulations that ensure high growth in high quality cell inoculum for the production bioreactors. Increase in time integral viable cell concentration (IVCC) is an area for media development targeting for prolonging culture longevity, increasing specific growth rate, and maximizing viable cell density [81]. The feed media for N-1 fed-batch stage require richer nutrients than routine growth media in order to sustain high viable cell density and balanced with components that contribute minimal level of toxic metabolites, such as lactate and ammonium.

Media for perfusion preparation

In the N-1 perfusion and intensification step, the goal in maintaining high quality of the cell inocula and steady productivity through the removal of potential toxic metabolites with medium exchanges, and thereby achieving high cell mass. The challenge,

Key term

Medium development: Process by which critical nutrients necessary for mammalian cell growth and protein production are combined in final formulations. The formulations may exist in a powder format that requires hydration before use or in a liquid format that can be used immediately.

however, will be controlling media cost and volume exchanges.

Media for N-stage production

Medium development for N-stage fed-batch production is designed to improve specific productivity by: enhancing biosynthetic pathway for therapeutic protein production through nutrient supplements necessary for cell proliferation and protein translation, maintaining favorable metabolic pathways that enhance cell performance and ensuring newly synthesized therapeutic proteins are efficiently traversed through secretory pathway and released into the extracellular milieu. Fed-batch production efficiency is driven by feed media and process control strategies to generate the final production titers. The formulations of production media and feeds tend to be highly enriched in order to support biosynthesis of proteins through the entire production stage. As technologies for studies at the genomic, transcriptomic, proteomic and metabolomic levels have been more prevalent; cell culture scientists are leveraging these tools to gain a better understanding in cellular physiology. The availability of the CHO genome database provides a direct path to re-engineering of the CHO cells for improved cellular characteristics [82–84]. Metabolite balance in high production cell culture can be achieved through a combination of metabolic flux and metabolite analyses [85,86]. Microarray and proteomics expression profiles have identified cellular proteins that are involved in regulating cell growth and viability [87,88]. A complete molecular profile of the CHO culture generated from a combination of these omics-based approaches will contribute significant cellular understanding of CHO cells, and provide a better path for predictive cell culture performance.

Another challenge to these highly nutrient enriched formulations is solubility of the media and feeds. The highly concentrated formulations not only need to be readily solubilizable from powder but also maintain as stable liquids upon hydration. Additional challenge is that drug manufacturers would prefer single or a few solution types in neutral pH to reduce operational complexity. While it is typical that multiple medium and feed solutions with both high and low pH are used during early media development and may be manageable at small scale, this approach is not compatible to a streamlined single feed solution strategy, and would demand comprehensive mixing studies to ensure these supplements are introduced appropriately prior to implementing at large-scale. To address these concerns, a number of laboratories have developed concentrates of chemically defined media (with solubility >100 g/l) by supplementing surfactants, such as polysorbates (PS80, PS20, P188) to the formulations [89,90]. Enhanced amino acid solubility in media was also achieved with amino acid analogs in the forms of peptides [91,92] and modified amino acids [93]. The ongoing development trend for highly concentrated feed medium formulations includes to refine and develop media concentrates at neutral pH that can be supplemented into the culture without significantly volume or osmolality increase to the final production batch, and to reduce the number of liquid formulations to enhance drug manufacture operations.

A strategic consideration in the medium development should also include a patent review on components in medium formulations as well as component uses associated with production processes. These challenges may require modification to medium design and process development.

Conclusion & future perspective

Future medium development for the next five to 10 years will be driven by drug demand, manufacturing process development strategy and facility selection. Biopharmaceutical manufacturers are considering facilities of modular designs having plug-and-play functionalities where different process steps are interchangeable. The drivers for this flexibility concept are to support the growth of personalized medicine, producing smaller batches, decentralize single manufacturing location, enabling modules to be deployed regionally, allow frequent new technology introduction, implementing new process improvements in plug-and-play modules that do not interfere with other modules and enable multiproduct manufacturing, maximizing facility capacity and reducing overall costs [94–96]. Flexibility in manufacture capability emphasizes agile facility having small footprint requirement, reduced location restriction, short set up time to support rapid manufacture of critical medicine [97]. Platform media and platform manufacturing processes are expected to be a part of the modular manufacture design. Future media would be in modular platform units to meet the production of multiple therapeutic products. The media and feeds would need to be highly stable concentrated liquids and easily solubilizable powder formulations that are insensitive to environmental fluctuation. Current development strategies to meet this demand should focus on better understanding of cellular responses to differences in medium components on biomolecule productivity.

Financial & competing interests disclosure

The author is an employee of Merck & Co. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Executive summary

Cell culture medium development history

- Cell culture media support Chinese hamster ovary (CHO) and other mammalian cell culture for continued propagation and protein production. Early in history medium components included complex animal-derived supplements to protect the production cells from environmental stress during manufacture. However, safety concerns due to contamination from adventitious agents in medium components have limited their usage. Chemically defined, animal-material-free components are preferred for biopharmaceutical manufacture today.

Chemically defined (CD) medium development strategies

- In addition to including components such as amino acids, vitamins, lipids, trace metals, salts and buffers in the CD formulations, the developed CD media should support cell line stability, improve overall cellular physiology and metabolism, meet protein quality expectation, as well as ensure medium manufacturability.

Medium development driven by protein production strategies

- The developed CD media should be compatible to production strategies. Common 'platform' media may be applicable for rapid therapeutic protein production. Complex production processes would necessitate significant medium development efforts.

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