Review
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Metabolism-guided design of serum-free media for modern biomanufacturing: from component control to cell-type-specific process optimization

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Abstract: Serum-free media (SFM) and chemically defined media (CDM) are increasingly important in modern biomanufacturing, reducing dependence on undefined serum components and improving raw-material control, reproducibility, and biosafety. However, rational medium development extends beyond replacing serum with defined supplements. This review presents SFM design as a metabolism-guided optimization problem in which nutrient composition, waste-metabolite accumulation, redox balance, lipid homeostasis, and growth-factor signaling jointly determine cell growth, product quality, and process robustness. We trace the evolution and classification of SFM/CDM and map amino acids, carbon sources, vitamins, lipids, trace elements, and signaling factors to the metabolic constraints they regulate. We compare cell-type-specific requirements across Chinese hamster ovary (CHO) and human embryonic kidney 293 (HEK293) production cells, mesenchymal stromal cells, induced pluripotent stem cells, chimeric antigen receptor T (CAR-T) and natural killer cells, primary-cell and organoid systems, and viral production platforms. We further examine how metabolomics, high-throughput screening, machine learning, and process analytical technologies support dynamic medium adjustment and closed-loop control. Finally, we consider economic, scalability, regulatory, and sustainability constraints. This framework links measurable cellular states to cell-type-specific medium formulation and feeding strategies for biologics and cell-based manufacturing.

Key words: Serum-free medium; Chemically defined medium; Metabolism-guided optimization; Metabolomics; Biomanufacturing; Cell therapy

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1 Introduction

Serum-free medium (SFM) emerged in the 1970s and has progressively reduced reliance on serum-dependent culture in biopharmaceutical manufacturing. Serum-free and chemically defined formulations are increasingly favored when raw-material traceability, process consistency, and adventitious-agent control are critical. However, adoption remains product-, cell-type-, and jurisdiction-dependent (Kang et al., 2024; US Food and Drug Administration [FDA], 2024a; Yang et al., 2025a). Therefore, low-serum or protein-containing serum-free formulations remain in use in selected applications when adaptation, performance, or cost constraints preclude immediate conversion to a fully chemically defined process. More precise control of basal nutrients, growth factors, and recombinant proteins can reduce batch-to-batch variability and contamination risk while decreasing reliance on animal-derived serum (Weber et al., 2025). Chemically defined media (CDM) have improved standardization in biologics manufacturing, but scale-up remains constrained by cell adaptation and medium cost. This review traces the evolution of SFM from basic definitions and classifications to its current industrial applications and identifies existing limitations. Unlike previous reviews that primarily catalog SFM components, classify animal-component-free formulations, or summarize specific application areas, this review uses metabolic control as the organizing framework (Ritacco et al., 2018; Combe and Sokolenko, 2021). We define "metabolism-driven optimization" as the rational adjustment of medium composition according to measurable cellular states, including nutrient uptake, metabolic flux, waste-metabolite accumulation, redox pressure, lipid homeostasis, and product-quality attributes (Templeton et al., 2013; Konakovsky et al., 2016; Schinn et al., 2021). This perspective incorporates basal formulation, dynamic feeding, cell-type-specific requirements, and process analytical technologies into a unified design logic. Accordingly, the central question is not which universal SFM formulation is optimal, but which metabolic limitation constrains a given cell system and how that limitation can be monitored and corrected (Schinn et al., 2021; Hashizume and Ying, 2025; Narayanan et al., 2025).

2 Definition and classification

SFM is a broad concept originally referring to a synthetic formulation that supports in vitro cell growth without animal serum. Distinct from natural media and basic synthetic formulations, SFM comprises a basal medium and targeted supplements. Typical serum-free formulations may contain dozens of defined nutrients and supplements, including amino acids, carbohydrates, lipids, growth factors, vitamins, and trace elements. These meet the metabolic requirements for cell proliferation and survival; additional supplements may be added based on specific cell-type requirements. According to compositional complexity and standardization level, SFM fall into four categories, as described below.

In practice, SFM eliminates serum but often retains animal-derived proteins such as albumin or select growth factors, making it a transitional formulation. At a more stringent standard, protein-free medium (PFM) excludes all animal-derived proteins and replaces them with plant protein hydrolysates. This avoids xenogeneic contamination while meeting cellular nutritional requirements. Animal-component-free medium (ACFM)

goes even further, excluding all animal-derived constituents and relying solely on recombinant or synthetic substitutes, an approach reserved for the most stringent ethical and regulatory scenarios. CDM represents a fully transparent formulation in which every ingredient has a defined chemical structure and molecular weight, thereby excluding undefined serum components, animal proteins, and complex plant hydrolysates. Because every component is specified precisely, batch variation drops to a minimum, placing CDM at the apex of the classification framework shown in Fig. 1 and making it particularly suitable for clinical-grade biomanufacturing and regulatory submissions.

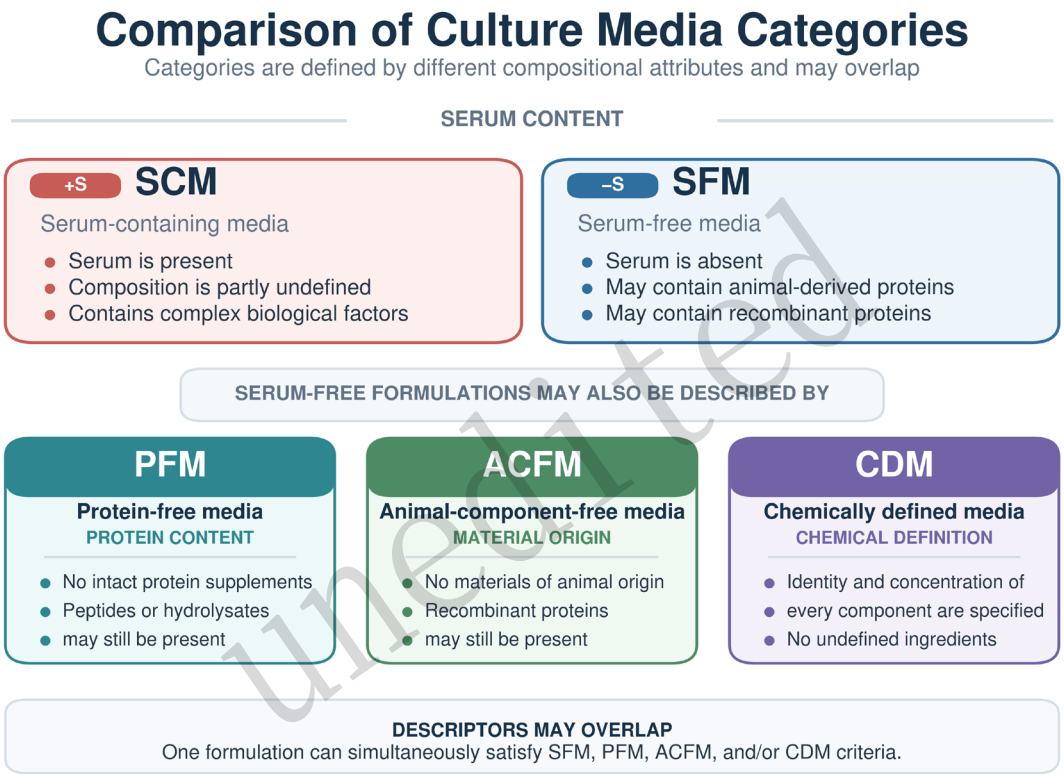

Fig. 1 Comparison of serum-containing and serum-free culture media categories. Serum-containing media use serum as a complex supplement, whereas SFM excludes serum but may retain proteins or other components of animal origin. PFM, ACFM, and CDM describe protein content, material origin, and degree of chemical definition, respectively. These categories may overlap and should not be interpreted as a strictly linear hierarchy. Abbreviations: SCM, serum-containing medium; SFM, serum-free medium; PFM, protein-free medium; ACFM, animal-component-free medium; CDM, chemically defined medium.

These descriptors should therefore be selected based on the property being reported—serum exclusion, protein content, material origin, or chemical definition—rather than used interchangeably as indicators of formulation stringency. The appropriate formulation ultimately depends on the cell type, product-quality requirements, process performance, and regulatory context.

3 Historical development and current situation

Harry Eagle established the foundation for modern cell culture media in 1955 by identifying the minimum essential nutrients required for in vitro cell growth (Eagle, 1955). He refined his initial formulation, which required >10% serum supplementation, into Minimal Essential Medium (MEM) in 1959. Early serum-dependent methods raised ethical concerns, incurred high costs, and involved batch-to-batch variability that compromised experimental reproducibility. An early milestone toward chemically defined culture was Ham's F-12 medium, reported in 1965 for the clonal growth of mammalian cells in a chemically defined synthetic medium (Ham, 1965). In 1976, Hayashi and Sato showed that defined hormones together with transferrin could replace serum and support cell growth in defined media (Hayashi and Sato, 1976). During the 1960s–1980s, research increasingly focused on identifying defined serum substitutes and optimizing nutrient concentrations. In 1982, Murakami et al. identified that ethanolamine is essential for hybridoma proliferation and achieved long-term growth in a defined medium supplemented with insulin, transferrin, ethanolamine, and selenium (ITES) (Murakami et al., 1982). Subsequently, medium development increasingly emphasized serum-free, animal-component-free, and chemically defined formulations to reduce dependence on undefined biological materials and to improve reproducibility (van der Valk et al., 2010). Modern SFM/CDM formulations can support high cell densities and recombinant protein production, although performance remains cell-line- and process-dependent. Metabolomic measurements are increasingly used to identify nutrient limitations and inhibitory by-products during formulation development. These advances have expanded the use of defined media in commercial biomanufacturing, where raw-material consistency and regulatory traceability are crucial process requirements (Ritacco et al., 2018; Combe and Sokolenko, 2021).

Beyond historical improvements in reproducibility and biosafety, current SFM/CDM adoption is driven by the manufacturing needs of biologics, cell and gene therapies, and other advanced bioprocesses. Rather than relying on market-size estimates from commercial reports, this review focuses on peer-reviewed evidence showing that advanced therapy manufacturing requires standardized, animal-component-free, and supply-chain-resilient raw materials (Ten Ham et al., 2018; Qiu et al., 2021). The COVID-19 pandemic further exposed vulnerabilities in biological raw-material supply chains and reinforced the importance of qualified, traceable, and regionally redundant materials for cell-therapy and biomanufacturing workflows (Cundell et al., 2020; Qiu et al., 2021). In parallel, recombinant production of growth factors, including FGF2, IGF1, PDGF-BB, and TGF-β1, is emerging as an enabling strategy for reducing animal-derived components and improving batch-to-batch consistency (Venkatesan et al., 2022). The central challenge has therefore shifted from serum replacement to metabolic control. Defined formulations must coordinate nutrient utilization with redox, ionic, membrane, and secretory homeostasis while limiting lactate, ammonium, and amino-acid-derived inhibitors and preserving cell-specific and product-quality attributes. The following section therefore discusses medium components, not as market-defined product categories but as metabolic levers that reshape cellular phenotype and process performance (Templeton et al., 2013; Konakovsky et al., 2016; Combe and Sokolenko, 2021).

4 Core components and functions

In a metabolism-guided framework, medium inputs act through interconnected control modules that collectively determine cellular state and process outcomes (Fig. 2). Carbohydrates and amino acids primarily

shape carbon and nitrogen metabolism; vitamins, cofactors, trace elements, and salts support cofactor availability, ionic balance, and redox homeostasis; lipids and delivery systems affect membrane organization and secretory trafficking; and hormones and growth factors regulate survival, proliferation, and cell identity (Ritacco et al., 2018; Combe and Sokolenko, 2021). These modules jointly influence energy and redox balance, biosynthetic capacity, stress responses, and secretory capacity, which are reflected in growth and viability, productivity, product quality, and cell-specific function. Lactate, ammonium, and amino-acid-derived inhibitors can accumulate as process-dependent metabolites and feed back negatively on cellular performance (Konakovsky et al., 2016; Ladiwala et al., 2023). The relationships shown in Fig. 2 represent principal contributions rather than exclusive pathways, as their direction and magnitude depend on the cell type, clone, basal formulation, culture mode, and process stage.

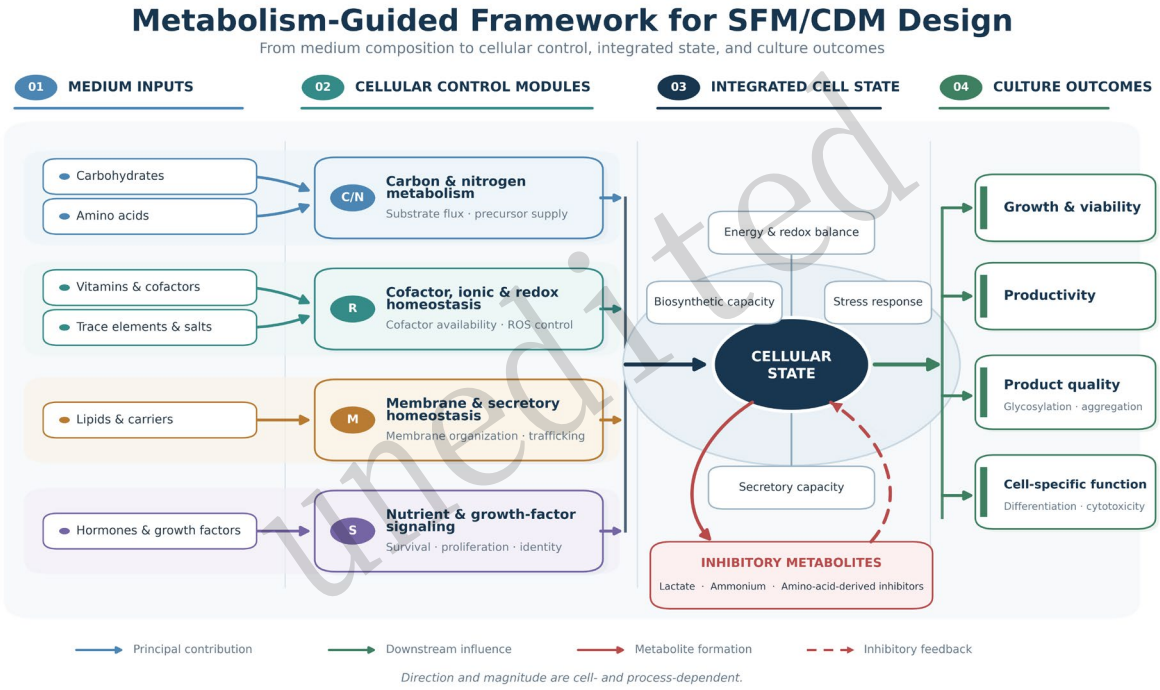

Fig. 2 Metabolism-guided framework for SFM/CDM design. Medium inputs act through four interacting control modules—carbon and nitrogen metabolism; cofactor, ionic, and redox homeostasis; membrane and secretory homeostasis; and nutrient- and growth-factor signaling—to shape an integrated cellular state defined by energy and redox balance, biosynthetic capacity, stress response, and secretory capacity. This state influences growth and viability; productivity; product quality, including glycosylation and aggregation; and cell-specific function, including differentiation and cytotoxicity. Lactate, ammonium, and amino-acid-derived inhibitors are shown as process-dependent metabolites that can feed back negatively on cellular state. Input-to-module arrows indicate principal contributions, green arrows indicate downstream influence, the solid red arrow indicates metabolite formation, and the dashed red arrow indicates inhibitory feedback. Direction and magnitude are cell- and process-dependent. Abbreviations: SFM, serum-free medium; CDM, chemically defined medium.

Reported concentration ranges in SFM and CDM should be interpreted as experimentally defined operating windows rather than universal formulation rules. Their transferability is constrained by cell lineage, clone, culture mode, basal formulation, delivery system, and endpoint, including growth and viability; productivity; product quality, such as glycosylation or aggregation; and cell-specific function, such as differ-

entiation or cytotoxicity (Reinhart et al., 2015; Combe and Sokolenko, 2021; Li et al., 2021). For example, commercial Chinese hamster ovary (CHO) media benchmarking shows that different host backgrounds and medium platforms can favor biomass accumulation or antibody production to different extents, indicating that a concentration beneficial for one CHO process may not optimize another (Reinhart et al., 2015; Combe and Sokolenko, 2021). Similarly, trace-metal supplementation illustrates endpoint dependence: manganese, copper, zinc, and selenium may regulate glycosylation or redox balance, but excessive or poorly controlled metal availability can impair growth or induce stress (Graham et al., 2019; Markert et al., 2020). Thus, metal concentrations should be reported alongside basal-medium composition, chelator/carrier context, and product-quality readouts (Graham et al., 2019; Markert et al., 2020). Accordingly, this review reports concentration values as context-specific examples and emphasizes structured optimization using metabolomics, high-throughput screening, or model-guided design when cross-study data conflict (Wells et al., 2020; Schinn et al., 2021; Narayanan et al., 2025).

4.1 Basal medium components: modulation of metabolic control modules

4.1.1 Amino acids: beyond nutrition—tools for metabolic engineering

Amino-acid supplementation in chemically defined media extends beyond biomass supply because extracellular stoichiometry determines both productive metabolism and the accumulation of inhibitory by-products. Direct quantitative evidence comes from a design of experiments (DOE)-guided CHO-K1 study using the AMBIC 1.0 reference medium. Lysine, isoleucine, tryptophan, leucine, and arginine were identified as key contributors to inhibitory-metabolite accumulation. As shown in Fig. 3, reducing their initial concentrations by 13%–33% and decreasing the fed-batch feed rate from 10% to 5% (v/v) reduced 2-hydroxyisocaproic acid (HICA) and N-acetylputrescinium (NAP) levels by up to 50%, methylsuccinic acid (MSA) by 30%, and cytidine monophosphate (CMP) by 15% (Ladiwala et al., 2023). The modified batch process increased peak viable-cell density and IgG titer by 13% and 7%, respectively, whereas the optimized fed-batch combination increased these endpoints by 40% and 50% (Ladiwala et al., 2023). These results demonstrate that amino-acid supply is not a universally monotonic input: reducing selected nutrients can improve both growth and productivity by preventing inhibitory-metabolite accumulation. Complementary genetic studies targeting BCAT1 or downstream BCKDHA/BCKDHB further support the importance of branched-chain amino acid (BCAA)-catabolic by-products and show that growth, viability, and specific productivity should be evaluated together (Lam et al., 2024).

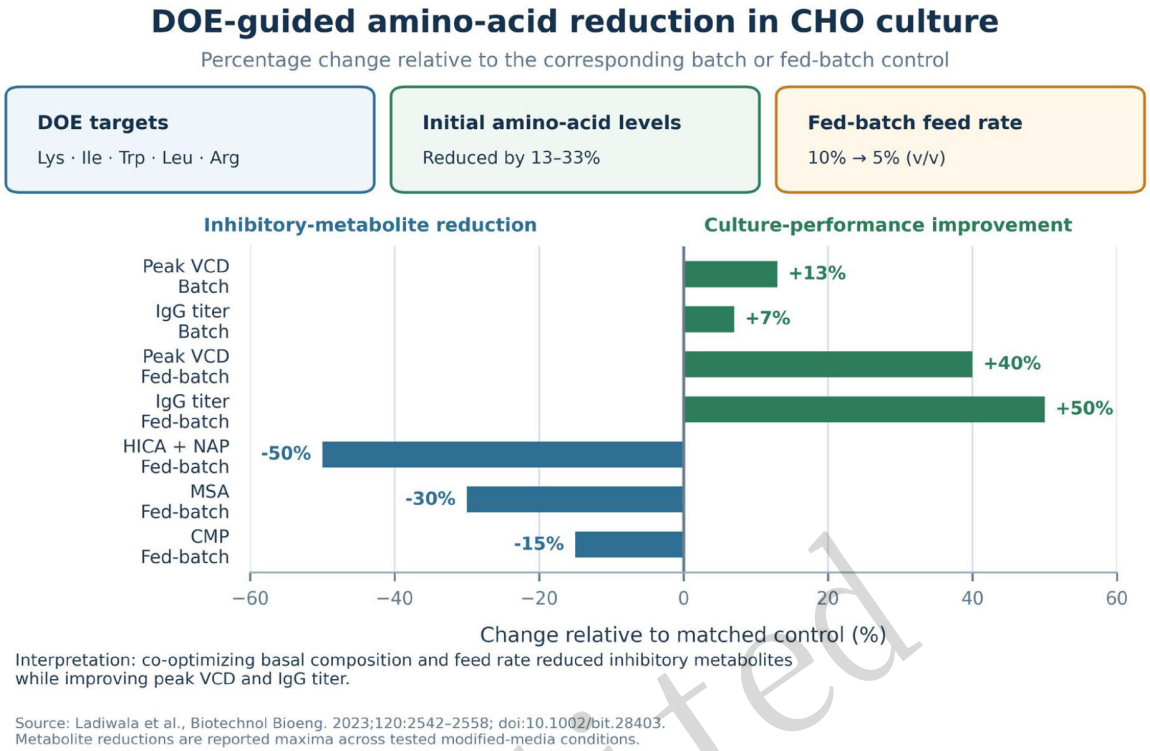

Fig. 3 Quantitative impact of DOE-guided amino-acid reduction on CHO culture performance. A response-surface design identified Lys, Ile, Trp, Leu, and Arg as key medium variables. Reducing their initial levels by 13%–33% and, in the optimized fed-batch condition, lowering the feed rate from 10% to 5% (v/v) reduced inhibitory-metabolite accumulation while increasing peak viable-cell density and IgG titer. Values represent percentage changes relative to the corresponding batch or fed-batch control; HICA/NAP, MSA, and CMP values are the maximum reductions reported across the tested modified-media conditions. Data were extracted and replotted from Ladiwala et al. (2023). Abbreviations: HICA, 2-hydroxyisocaproic acid; NAP, N-acetylputrescinium; MSA, methylsuccinic acid; CMP, cytidine monophosphate; VCD, viable cell density.

While individual amino acids can affect culture performance through mechanisms beyond protein synthesis, these effects should not be generalized across amino acids or cell lines. Histidine can contribute to extracellular buffering due to its imidazole side chain, whereas lysine contains an ε-amino group and should not be described as an imidazole buffer. Separately, medium composition can alter nucleotide-sugar availability, thereby influencing N-glycan galactosylation; these effects should be attributed to the specific carbon or precursor supplementation tested in the study by Pulipeta et al. (2025).

4.1.2 Vitamins: cofactor supply, redox control, and stability

In serum-free and chemically defined media, B vitamins primarily serve as enzyme cofactors or cofactor precursors, whereas choline and myo-inositol are vitamin-like components that support membrane and phosphoinositide metabolism. Thiamine, riboflavin, niacinamide, pantothenate, and pyridoxine support TPP-, FAD/FMN-, NAD(P)-, CoA-, and PLP-dependent reactions, respectively; biotin supports carboxylation, while folate and cobalamin support one-carbon and methionine metabolism (Schnellbaecher et al., 2019). Table 1 summarizes traceable formulation values, a platform-specific human pluripotent stem cell (hPSC) example, and a reported CHO vitamin-fortification screening window. The B-vitamin, choline chloride, and i-inositol

concentrations are the values reported for Gibco DMEM/F-12, product 11320 (Thermo Fisher Scientific, n.d.); the ATCC DMEM: F-12 formulation (ATCC 30-2006) was used only as an independent rounded composition cross-check (American Type Culture Collection n.d.). These concentrations are traceable formulation references rather than universal optima, as requirements vary with cell line, basal formulation, culture mode, feed strategy, and process endpoint (Schnellbaecher et al., 2019; Combe and Sokolenko, 2021; Hashizume and Ying, 2025). In two recombinant CHO cell lines, 13 vitamins were evaluated individually using the basal-medium concentration (1×) and a twofold-fortified condition (2×). Choline chloride, folic acid, thiamine hydrochloride, myo-inositol, niacinamide, and pyridoxine hydrochloride produced clone-dependent improvements in cell growth and/or antibody production, whereas twofold pyridoxal hydrochloride supplementation inhibited growth in both cell lines (Kim et al., 2005). Consequently, 1×–2× of the basal formulation represents a reported initial screening window for individual vitamins in CHO cells, rather than a universally recommended concentration range.

Process-specific evidence reinforces this distinction. In a CHO fed-batch study, thiamin could be removed from the feed without affecting the tested cell lines when basal-medium supply was sufficient (Schnellbächer and Zimmer, 2023); conversely, choline limitation reduced cell viability and monoclonal antibody (mAb) titer and increased aggregate and mannose-5 contents in a defined CHO platform (Kuwae et al., 2018). In hPSC culture, E8 contains 64 mg/L L-ascorbic acid 2-phosphate magnesium salt, a stabilized ascorbate derivative (Chen et al., 2011). This platform-specific mass concentration should not be generalized to CHO, HEK293, or immune-cell processes. Vitamin stability is also a formulation variable: under UVA exposure, riboflavin-mediated photochemistry can generate reactive oxygen species, and riboflavin can promote the photodegradation of folate species (Steindal et al., 2008; Schnellbaecher et al., 2019; Yoshimoto et al., 2020). Light-sensitive formulations should therefore be protected during preparation, storage, and feeding, and vitamin modules should be optimized jointly with trace metals and other redox-active components through structured, cell-specific studies rather than isolated dose-escalation experiments (Schnellbaecher et al., 2019; Combe and Sokolenko, 2021; Hashizume and Ying, 2025).

Table 1 Representative vitamin types, reported concentrations, and formulation considerations in serum-free and chemically defined media

Vitamin module	Representative reference level	Key function and practical considerations
Energy/redox and amino-acid cofactor module (B1, B2, B3, B5, B6)	Gibco DMEM/F-12 (11320): B1 6.44; B2 0.582; B3 16.56; B5 4.70; B6 9.77 μmol/L	Supports TPP-, FAD/FMN-, NAD(P)-, CoA-, and PLP-dependent reactions. Use basal levels as traceable starting references; additional feed requirements should be evaluated in the target cell line and light-sensitive components should be protected (Schnellbaecher et al., 2019; Yoshimoto et al., 2020; Schnellbächer and Zimmer, 2023; American Type Culture Collection n.d.; Thermo Fisher Scientific, n.d.).

Vitamin module	Representative reference level	Key function and practical considerations
One-carbon and carboxylase module (B7, B9, B12)	Gibco DMEM/F-12 (11320): B7 0.0143; B9 6.01; B12 0.502 μmol/L	Supports carboxylation, one-carbon transfer, nucleotide synthesis, and methionine metabolism. Riboflavin can promote folate photodegradation; light exposure should be controlled during preparation and storage (Steindal et al., 2008; Schnellbaecher et al., 2019; American Type Culture Collection n.d.; Thermo Fisher Scientific, n.d.).
Membrane/phospholipid-related vitamin-like module (choline and myo-inositol)	Gibco DMEM/F-12 (11320): choline chloride 64.14 μmol/L; i-inositol 70.0 μmol/L	Supports phospholipid synthesis and phosphoinositide signaling. Choline demand is process-dependent and should be optimized together with basal and feed composition (Kuwae et al., 2018; American Type Culture Collection n.d.; Thermo Fisher Scientific, n.d.).
Platform-specific stabilized vitamin C example	E8 hPSC medium: L-ascorbic acid 2-phosphate magnesium 64 mg/L	A defined hPSC-platform example. The chemical form should be reported explicitly, and this mass concentration should not be generalized to CHO, HEK293, or immune-cell processes (Chen et al., 2011; Schnellbaecher et al., 2019; Thermo Fisher Scientific, n.d.).
CHO vitamin-fortification screen	Each of 13 vitamins was tested individually at 1× and 2× its basal-medium concentration	Responses were clone-dependent; 2× pyridoxal hydrochloride inhibited growth in both tested cell lines. The basal composition and chemical form must therefore be reported (Kim et al., 2005).

Note: Concentrations of B vitamins, choline chloride, and i-inositol were obtained from the official formulation of Gibco DMEM/F-12 (Product No. 11320) (Thermo Fisher Scientific, n.d.). The ATCC DMEM: F-12 formulation (ATCC 30-2006) (American Type Culture Collection n.d.) was used as an independent cross-check of the reported composition; values from the two formulations were not averaged. Concentrations in μM were calculated from the chemical forms and molecular weights specified in the selected Gibco formulation. The 64 mg/L L-ascorbic acid 2-phosphate magnesium value was obtained from the E8 hPSC formulation reported by Chen et al. (2011) and should not be interpreted as a universal concentration for other cell systems. In the CHO vitamin-fortification study, 1× and 2× refer to the basal and twofold concentrations, respectively, of each individual vitamin in the study-specific medium; these relative levels should not be converted directly using the DMEM/F-12 concentrations listed above (Kim et al., 2005).

4.1.3 Inorganic salts: hidden switches for mechanotransduction

Beyond osmotic regulation, specific ionic ratios in CDM serve as mechanotransduction signals. Systematic quantification of chemically defined media components shows that Ca^{2+}/Mg^{2+} molar ratios and phosphate buffer concentrations significantly affect CHO cell aggregation dynamics, shear-stress tolerance, and cultivation stability, parameters that are particularly critical in industrial bioreactor operations where mechanical stress correlates with cell viability (Combe and Sokolenko, 2021).

4.2 Functional additives: modulating cellular phenotypes

4.2.1 Carbohydrate metabolism: engineering oxidative phenotypes

Rather than serving solely as an energy source, glucose availability influences cellular metabolism. In one CHO study, simultaneously replacing glucose and glutamine with the more slowly metabolized substrates galactose and glutamate supported cell growth while minimizing lactate and ammonium accumulation (Altamirano et al., 2000). Separately, time-dependent galactose-manganese feeding modulated intracellular UDP-galactose availability and monoclonal-antibody glycosylation (Gyorgypal et al., 2025). Pyruvate may also provide metabolic and redox protection in selected cell models, but its effects should not be generalized across serum-free culture systems (Hegde et al., 2010).

4.2.2 Protein carriers: regulating iron transport dynamics

Recombinant albumin and transferrin require concentration optimization based on iron saturation kinetics. Benchmarking studies demonstrate that subtle variations in these proteins significantly affect CHO cell growth and antibody productivity (van der Valk et al., 2010; Brühlmann et al., 2015), indicating that precise titration is preferable to empirical supplementation.

4.2.3 Lipids and lipid-delivery systems: membrane homeostasis and secretory function

Lipids in SFM/CDM include cholesterol, free fatty acids, phospholipids, and sphingolipids, which regulate membrane order, lipid microdomain organization, and secretory trafficking (Ritacco et al., 2018; Levental et al., 2020). Their requirements depend on cell lineage, endogenous sterol synthesis, culture format, extracellular matrix, and delivery vehicle. In a defined adherent cancer-cell model, 30 μmol/L soluble cholesterol delivered as a methyl-β-cyclodextrin complex modified membrane cholesterol, and both cholesterol addition and depletion impaired cell adhesion (Takii et al., 2022). Conversely, lipid-free medium supplemented with 1× insulin-transferrin-selenium supported the proliferation of some—but not all—melanoma cell lines, indicating variable dependence on exogenous lipids (Wu and Näär, 2019). In chemically defined CHO medium, 26 fatty acids were screened at 10 and 20 μmol/L; EDA showed the strongest response at 10 μmol/L, with reduced benefit at higher concentrations and exhibiting dependence on the timing of addition (Pei et al., 2026). In human embryonic stem cells (hESCs) cultured in N2/B27-CDM, LPC, LPA, and S1P were tested at 100, 10, and 3–10 μmol/L, respectively; 10 μmol/L S1P caused marked cell death, whereas 3 μmol/L was better tolerated (Garcia-Gonzalo and Izpisúa Belmonte, 2008). Collectively, these reports show that lipid supplementation is strongly model-dependent and that the concentrations listed in Table 2 should not be treated as universal recommendations.

Table 2 Representative lipid conditions and reporting considerations in serum-free media

Lipid module	Representative reported conditions	Interpretation
Cholesterol	30 μmol/L soluble cholesterol delivered as an MβCD complex in adherent cancer cells	Both cholesterol addition and depletion impaired adhesion in the tested model (Takii et al., 2022)
Lipid-free condition	1× ITS without exogenous lipids in melanoma culture	Supports selected cell lines with sufficient de novo lipogenesis (Wu and Näär, 2019)

Lipid module	Representative reported conditions	Interpretation
Free fatty acids in CHO cells	Twenty-six fatty acids screened at 10 and 20 μmol/L; EDA showed the strongest response at 10 μmol/L	EDA showed the strongest response at 10 μmol/L; fatty-acid species, solvent, and addition time should be reported (Pei et al., 2026)
Lysophospholipids/sphingolipids in hESCs	LPC 100 μmol/L; LPA 10 μmol/L; S1P 3–10 μmol/L	10 μmol/L S1P caused marked toxicity, whereas 3 μmol/L was better tolerated (Garcia-Gonzalo and Izpisúa Belmonte, 2008)

Note: The listed concentrations represent model-specific screening, supplementation, or perturbation conditions and should not be treated as universal optima. Lipid species, chemical form, solvent or delivery vehicle, addition time, cell type, and experimental endpoint should be reported. MβCD, methyl-β-cyclodextrin; ITS, insulin-transferrin-selenium; LPC, lysophosphatidylcholine; LPA, lysophosphatidic acid; S1P, sphingosine-1-phosphate; EDA, cis, cis-11,14-eicosadienoic acid.

4.2.4 Hormones and growth factors: dose-dependent signal modulation

In CDM, hormones and growth factors act as defined signaling inputs that support cell survival, proliferation, and recombinant-protein production, but their effects depend on the cell line, basal formulation, and culture stage. Insulin is widely used in serum-free CHO culture; however, a direct comparison between two recombinant CHO cell lines showed that the IGF-1 analog LongR3 IGF-I maintained higher viability than insulin under production conditions, and this improved viability was accompanied by higher product titers (Morris and Schmid, 2000). Growth-factor effects can also depend on combinatorial context. In M-CSF-producing CHO cells and CHO-K1 cells cultured in basal DMEM/F12, insulin or basic fibroblast growth factor (bFGF) alone maintained viability but did not restore proliferation, whereas combined supplementation synergistically promoted cell growth and recombinant M-CSF synthesis (Liu and Wu, 2009). These findings indicate that hormone and growth-factor modules should be optimized as cell-line- and process-specific combinations using viability, viable cell density, and product titer as joint readouts, rather than transferring a single concentration or dosing schedule across CHO processes (Morris and Schmid, 2000; Liu and Wu, 2009).

4.2.5 Other functional additives

Functional additives can improve the performance of SFM not only by supplying nutrients but also by preserving the activity of labile signaling proteins. FGF2 is intrinsically unstable under routine culture conditions, and its loss of activity can alter hPSC self-renewal and differentiation; therefore, growth-factor stability and replacement frequency should be treated as formulation variables (Chen et al., 2012). Protein engineering offers one solution: a disulfide-bond-stabilized thermostable bFGF retained activity for longer and supported better proliferation, stemness, morphology, and differentiation of hPSCs than wild-type bFGF (Kim et al., 2023). These findings support optimizing the molecular stability, storage, and dosing schedule of growth factors rather than attributing the effect to nonspecific protease inhibition (Chen et al., 2012; Kim et al., 2023).

Nucleotide-precursor supplementation can improve CHO cell growth in a context-dependent manner.

Morrison et al. evaluated two CHO clones and found that a combination of cytidine, hypoxanthine, uridine, and thymidine at 100 μM each significantly increased growth during routine passaging; the same combination also improved growth rate and harvest cell density in seed bioreactors (Morrison et al., 2019). However, in production fed-batch bioreactors, supplementation did not improve growth rate or peak viable cell density, and the study did not evaluate antibody glycosylation. Thus, nucleotide-precursor supplementation should be optimized separately for passaging, seed expansion, and production stages, and claims regarding glycosylation require direct product-quality measurements (Morrison et al., 2019).

Trace elements should be treated as context-dependent regulatory variables rather than fixed universal supplements. In CHO cultures, variability in the availability of zinc, copper, manganese, and iron can affect oxidative stress, apoptosis, lactate metabolism, productivity, and glycan synthesis, and the direction and magnitude of these effects depend on the basal formulation and cell line (Graham et al., 2019). Automated high-throughput screening combined with design of experiments has further shown that trace-element combinations can be optimized against monoclonal-antibody N-glycosylation endpoints rather than cell growth alone (Markert et al., 2020). Therefore, trace-metal concentrations should be established empirically while simultaneously monitoring viability, productivity, metabolism, and product-quality attributes; concentration ranges reported for one system should not be presented as universal optima (Graham et al., 2019; Markert et al., 2020).

5 SFM design and optimization strategy

Because component effects are nonlinear and interdependent, modern SFM optimization increasingly relies on systems-level measurements and model-guided experimentation. Metabolomics, metabolic flux analysis, genome-scale modeling, and machine-learning-assisted design can reduce the empirical search space by identifying limiting substrates, toxic byproducts, and actionable pathway bottlenecks (Templeton et al., 2013; Schinn et al., 2021; Narayanan et al., 2025). These approaches transform SFM development from additive-by-additive screening into iterative hypothesis generation, experimental validation, and feedback-controlled refinement (Schinn et al., 2021; Hashizume and Ying, 2025; Narayanan et al., 2025).

5.1 Metabolomics-driven formulation design

SFM design is shifting from experience-driven to data-driven approaches, with metabolomics and metabolic flux analysis providing a basis for identifying limiting substrates, inhibitory by-products, and stage-specific pathway demands. High-throughput mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy can quantify dynamic metabolite changes during cultivation and support the development of testable formulation hypotheses (McCloskey et al., 2016; Beale et al., 2018). In an industrially relevant fed-batch CHO process, ^{13}C metabolic flux analysis showed that peak cell growth was associated with high lactate production and limited tricarboxylic acid (TCA)-cycle activity, whereas the transition to peak antibody production was accompanied by net lactate consumption, increased TCA cycling, oxidative phosphorylation, and oxidative pentose-phosphate-pathway activity (Templeton et al., 2013). This phase-dependent metabolic rewiring illustrates why medium and feed interventions should be linked to culture stage and verified experimentally rather than inferred from a single metabolite measurement. Machine-learning models that integrate

metabolic and growth data can further prioritize amino-acid combinations and reduce the experimental search space (Schinn et al., 2021). Combined, these approaches support an iterative workflow comprising metabolic profiling, hypothesis generation, experimental validation, and formulation refinement (Templeton et al., 2013; Schinn et al., 2021).

5.2 High-throughput screening (HTS) and machine learning-driven optimization

High-throughput screening (HTS) enables the simultaneous evaluation of multiple formulation variables. Screening designs can first narrow the experimental search space, while response-surface methods can estimate component effects and interactions. Machine-learning and active-learning models can then prioritize subsequent formulations (Ladiwala et al., 2023; Hashizume and Ying, 2025; Hashizume et al., 2026). These approaches have also linked medium composition to product-quality attributes, including monoclonal-antibody N-glycosylation and charge variants (Markert et al., 2020; Gangwar et al., 2024).

Recent studies have shifted medium development from one-shot factorial screening to iterative, data-efficient optimization. Hashizume and Ying developed a biology-aware machine-learning platform that integrated error-aware data processing, predictive model construction, and active learning. In CHO-K1 cells, Hashizume and Ying experimentally evaluated 364 formulations to fine-tune a 57-component serum-free medium, and the reformulated medium achieved approximately 60% higher cell concentration than commercial alternatives (Hashizume and Ying, 2025). In a subsequent study, Hashizume et al. combined design of experiments with two machine-learning models in a sequential active-learning workflow for IgG-producing CHO cells. By iteratively adjusting 44 serum-free medium components and incorporating biological constraints, including osmolality and amino-acid composition, this strategy significantly improved IgG production with limited experimental resources (Hashizume et al., 2026). Beyond growth and antibody titer, Gangwar et al. developed an explainable artificial-intelligence framework for CHO-GS cultures that combined feature selection, random-forest methods, SHapley Additive exPlanations, and regression modeling to relate metal-ion composition to acidic and basic charge variants, identifying iron and zinc as influential formulation variables (Gangwar et al., 2024). Collectively, these studies show that structured experimentation, active learning, and explainable machine learning can make medium optimization more data-efficient while linking formulation variables to cell growth, antibody productivity, and product-quality attributes. Nevertheless, the resulting optima remain dependent on the cell line, basal medium, culture mode, and selected optimization objective (Gangwar et al., 2024; Hashizume and Ying, 2025; Hashizume et al., 2026).

5.3 Dynamic medium adjustment

Medium optimization extends beyond the initial formulation because nutrient demand and by-product formation change throughout culture. Batch culture is susceptible to nutrient depletion and metabolite accumulation. By contrast, fed-batch and perfusion strategies can use indicators such as viable cell density or online biomass measurements to adjust nutrient delivery or the cell-specific perfusion rate; suitable indicators and control rules must be validated for the specific cell line and process (Schulze et al., 2021; Kikuchi et al., 2025a; Walther et al., 2025).

Fed-batch culture uses the periodic or continuous addition of concentrated feeds to replenish substrates while avoiding the inhibitory effects of excessively high initial nutrient concentrations. Relevant control variables include feed composition, timing, and rate, which can be set based on specific consumption rates or ad-

justed using measured metabolite profiles (Konakovsky et al., 2016). Medium composition and host-cell metabolism provide complementary levers for limiting waste formation. In one CHO study, simultaneously replacing glucose and glutamine with the more slowly metabolized substrates galactose and glutamate supported cell growth while minimizing lactate and ammonium accumulation (Altamirano et al., 2000). Separately, PYC2 overexpression in an antibody-producing CHO line shifted metabolism toward lactate consumption, prolonged exponential growth, and increased maximum cell concentration and volumetric product titer (Toussaint et al., 2016). These results should be interpreted as distinct formulation and cell-engineering strategies rather than as evidence that alanine or pyruvate substitution is a general fed-batch solution (Altamirano et al., 2000; Toussaint et al., 2016).

Perfusion culture extends dynamic nutrient delivery to continuous operation, with the cell-specific perfusion rate (CSPR) as a key process variable linking medium flow to viable cell density. CSPR should be optimized for the specific cell line, medium, and process objective rather than assigned a universal operating range (Schulze et al., 2021; Walther et al., 2025). Very-high-density CHO perfusion has also been demonstrated with alternating tangential flow (ATF) and tangential flow filtration (TFF) systems for cell retention, underscoring that attainable density depends on the process and the device (Clincke et al., 2013). In a high-density perfusion study used for Raman-model development, CSPR values of 10–40 pL/(cell·day) were evaluated, with cell densities reaching up to 100×10^6 cells/mL; however, changes in CSPR substantially altered metabolism, monoclonal-antibody productivity, and N-glycosylation (Schwarz et al., 2022). In a separate process-intensification study, Stepper et al. used pre-stage TFF perfusion to reach up to 45×10^6 cells/mL and then inoculated an ultra-high-seeding-density fed-batch process. The intensified process produced a 1.9-fold higher titer than the low-seeded reference within an equivalent runtime and achieved comparable product quality (Stepper et al., 2020). Hiller et al. likewise reported that a short cell-controlled perfusion phase followed by conventional fed-batch approximately doubled overall productivity across five CHO monoclonal-antibody processes (Hiller et al., 2017). Together, these studies show that CSPR, cell density, medium consumption, and product quality must be optimized jointly, and case-specific values should not be presented as universal optima (Clincke et al., 2013; Hiller et al., 2017; Stepper et al., 2020; Schulze et al., 2021; Schwarz et al., 2022; Walther et al., 2025).

Advanced process analytical technologies can support both process monitoring and feedback-controlled feeding. In high-cell-density CHO perfusion cultures, Schwarz et al. varied CSPR from 10 to 40 pL/(cell·day) to generate calibration data and developed Raman-based predictive models for viable cell density, lactate, ammonium, multiple amino acids, and antibody N-glycosylation (Schwarz et al., 2022). The study demonstrated real-time prediction capability but did not implement automatic control of asparagine, glutamine, uridine, or manganese. By contrast, Domján et al. coupled Raman monitoring of arginine and glucose with the automated delivery of multicomponent and concentrated-glucose feeds in CHO culture (Domján et al., 2022). Matthews et al. used Raman-predicted glucose and lactate concentrations to control glucose feeding; maintain lactate at predefined setpoints; and improve culture duration, viability, and protein productivity (Matthews et al., 2016).

Collectively, these studies distinguish three stages of process analytical technology implementation: the prediction of process variables and product-quality attributes, the automated control of nutrient feeds, and the closed-loop regulation of inhibitory metabolites (Matthews et al., 2016; Domján et al., 2022; Schwarz et al., 2022). While Raman-based prediction of N-glycosylation is therefore a promising monitoring tool, the proac-

tive adjustment of glycosylation through uridine or manganese should only be claimed when those feed manipulations are directly tested. Model calibration, transferability across cell lines, and independent validation remain essential before using a process analytical technology model for process control (Matthews et al., 2016; Hebing et al., 2020; Domján et al., 2022; Espinel-Ríos et al., 2024).

Overall, dynamic feeding integrates metabolic monitoring with stage-specific nutrient delivery, bridging medium formulation and closed-loop process control.

6 Application of SFM in different cell types and scenarios

CHO and HEK293 platforms are often optimized around high-density growth, lactate/ammonium control, transfection efficiency, and product-quality attributes; mesenchymal stromal cell (MSC)/ induced pluripotent stem cell (iPSC) systems require the integration of soluble signals with extracellular matrix (ECM)-defined microenvironments, and CAR-T and NK-cell manufacturing must balance expansion with memory phenotype, exhaustion, redox state, and cytotoxicity (Badenes et al., 2016; Eberhardt et al., 2023; Muthuvel et al., 2025). Thus, cross-cell-type comparison aims not to rank universal nutrient requirements, but to identify the dominant metabolic and microenvironmental bottlenecks that guide formulation design. Fig. 4 compares four major serum-free and chemically defined culture platforms using four common decision layers: dominant biological constraints, medium-design priorities, decision readouts, and a platform-specific design principle. Th is qualitative comparison aims to identify platform-specific metabolic and microenvironmental bottlenecks rather than rank universal ingredient requirements or recommend generally applicable concentration ranges.

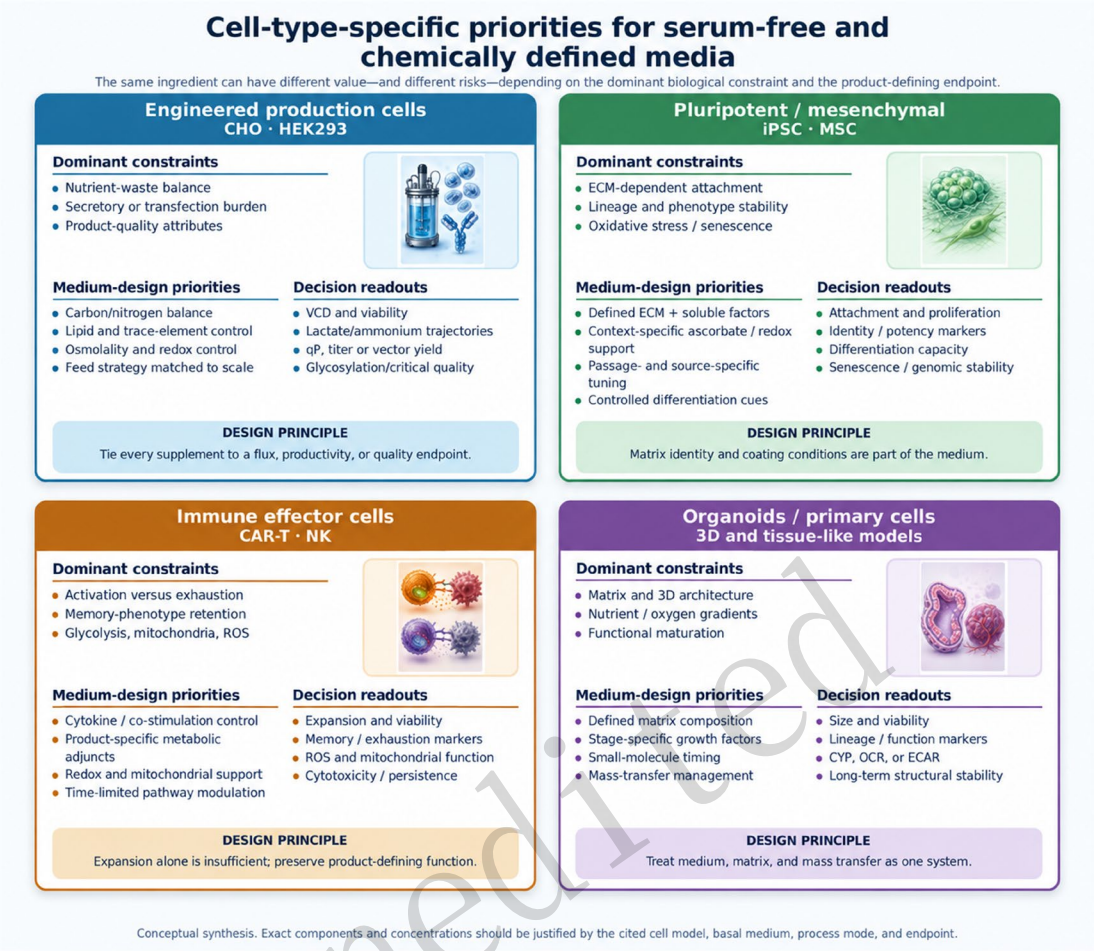

Fig. 4 Cell-type-specific priorities for serum-free and chemically defined media. Qualitative comparison of major cell platforms, highlighting platform-specific biological constraints, medium-design priorities, and decision readouts rather than universal formulation recommendations. Abbreviations: CHO, Chinese hamster ovary; HEK293, human embryonic kidney 293; iPSC, induced pluripotent stem cell; MSC, mesenchymal stromal/stem cell; CAR-T, chimeric antigen receptor T cell; NK, natural killer cell; ECM, extracellular matrix; ROS, reactive oxygen species; VCD, viable cell density; qP, cell-specific productivity; CYP, cytochrome P450; OCR, oxygen consumption rate; ECAR, extracellular acidification rate. Based on representative studies (Petiot et al., 2011; Badenes et al., 2016; Ojo et al., 2019; Combe and Sokolenko, 2021; Panella et al., 2021; Eberhardt et al., 2023; Harrison et al., 2023); additional supporting references are provided in Sections 6.1–6.4.

6.1 Industrialized application of engineering cell lines (CHO, HEK293)

In biopharmaceutical manufacturing, SFM/CDM design for engineered cell lines must reflect host-specific metabolic and process requirements. Human embryonic kidney 293 (HEK293) cells are widely used for recombinant-protein and viral-vector production. In a fed-perfusion process, high-cell-density transfection at 2×10^7 cells/mL and the optimization of plasmid DNA load, PEI-to-DNA ratio, and post-transfection feeding increased rAAV volumetric productivity to nearly 2×10^{12} vg/mL (Deng et al.,

2025). More broadly, animal-component-free and chemically defined HEK293 platforms are being developed for scalable transfection and production, although performance remains dependent on the vector or protein product and the selected culture process (Grieger and Samulski, 2012; Subedi et al., 2015; Davidsson et al., 2020; Fiol et al., 2023; Coplan et al., 2024; Zhang et al., 2024; Kowshik and Singh, 2025).

For CHO cells, which are the primary host system for therapeutic protein production, SFM development extends further toward precise modulation of cellular metabolism and control over product quality attributes (Combe and Sokolenko, 2021; Sahoo et al., 2024). These media contain carefully balanced amino acids, vitamins, trace elements, and lipids. This nutritional support facilitates high cell densities and extended viability in fed-batch or perfusion cultures (Kikuchi et al., 2025a). Additionally, these supplements shape post-translational modifications, particularly the glycosylation patterns that determine drug efficacy and pharmacokinetic behavior (Kranjc et al., 2024). Specifically, medium optimization for glycosylation control operates through three complementary mechanisms: First, precursor-directed glycoengineering—supplementation with nucleotide sugar precursors such as galactose, uridine, and N-acetylglucosamine—directly modulates intracellular pools of UDP-Gal and UDP-GlcNAc, enabling targeted enhancement of galactosylation and N-glycan branching without genetic modification (Wells et al., 2020; Pulipeta et al., 2025). Second, trace element precision—particularly manganese and copper—serves as a metabolic switch: manganese acts as an essential cofactor for β-galactosyltransferase and α-2,3-sialyltransferase, thereby promoting terminal galactosylation and sialylation, while copper concentration fine-tuning can redirect glycosylation maturation pathways independent of cell growth (Graham et al., 2019; Markert et al., 2020). Temporal optimization of these additives (e.g., stage-specific galactose feeding or manganese pulsing) further decouples glycosylation control from cell proliferation, enabling independent optimization of product quality attributes (Gyorgypal et al., 2025). Media design can also be combined with host-cell engineering to reduce specific metabolic bottlenecks or stress responses (Lim et al., 2025). In this approach, cell-line engineering and medium optimization are treated as complementary strategies rather than as sequential selection steps (Fletcher et al., 2026).

6.2 Stem cell and iPSC cultivation

For MSCs and iPSCs, serum-free culture requires co-definition of the soluble medium and the adhesion substrate. In hPSC systems, E8 medium together with recombinant vitronectin supports defined, xeno-free maintenance and scalable expansion; one reported microcarrier protocol used vitronectin at 0.5 μg/cm² (Chen et al., 2011; Badenes et al., 2016). Recombinant LN-521 alone was applied at 30 μg/mL (5 μg/cm²) by overnight coating at 4 °C, and the reported clonal-culture protocol used an LN-521/E-cadherin matrix containing 13.5 and 1.5 μg/mL, respectively (2.25 and 0.25 μg/cm²; 9:1 w/w), with coating for 2 h at 37 °C (Rodin et al., 2014). For MSCs, matrix responses remain dependent on tissue source and process conditions (Swamynathan et al., 2014; Panella et al., 2021). In one human bone-marrow MSC study, tissue-culture plastic was coated with fibronectin at 5 μg/mL and evaluated alongside a chemically defined xeno/serum-free expansion medium (Basoli et al., 2021). These concentrations are platform-specific reported conditions rather than universal optima. Table 3 summarizes representative reported two-dimensional ECM coating conditions. ECM identity, coating concentration or surface density, substrate, coating duration, cell source, and attachment, proliferation, phenotype, and differentiation endpoints should therefore be reported. For three-dimensional culture, matrix composition, protein or polymer concentration, and stiffness should be reported instead of a two-dimensional

coating density.

During extended or repeated passaging, redox status and cellular senescence should be monitored, and hPSC cultures also require surveillance for culture-acquired genetic changes (Sriram et al., 2019; Andrews et al., 2022). Ascorbate or other antioxidant supplementation should therefore be treated as source- and context-specific rather than as a universal SFM component (Sriram et al., 2019). These variables should be evaluated alongside attachment, proliferation, identity or potency, differentiation capacity, senescence, and genomic stability (Sriram et al., 2019; Andrews et al., 2022).

Table 3 Representative ECM coating conditions and reporting parameters for serum-free MSC and hPSC culture

Cell system	ECM example	Representative reported condition	Reporting focus
iPSC/hPSC	Recombinant vitronectin	0.5 μg/cm² on polystyrene microcarriers; coating for 2 h at room temperature	Specify substrate, coated area, coating duration and passage method (Badenes et al., 2016)
hPSC	Recombinant LN-521	30 μg/mL (5 μg/cm²); overnight coating at 4 °C	Supports attachment and self-renewal; this is not the clonal LN-521/E-cadherin condition (Rodin et al., 2014)
hESC clonal culture	Recombinant LN-521/E-cadherin	LN-521 13.5 μg/mL + E-cadherin 1.5 μg/mL (2.25 + 0.25 μg/cm²; 9:1 w/w); 2 h at 37 °C	Report single-cell dissociation, seeding density and clonal-survival endpoints (Rodin et al., 2014)
Bone-marrow MSC	Fibronectin	5 μg/mL fibronectin in PBS on tissue-culture plastic	Report MSC source, medium, attachment, proliferation, and differentiation endpoints (Basoli et al., 2021)

Note: These conditions are reported platform-specific examples rather than universal optima. For two-dimensional culture, both coating-solution concentration and surface density should be reported when available, along with substrate material, coating duration, temperature, cell source, and passaging method. For LN-521, the solution concentration and surface density should not be interchanged. The LN-521/E-cadherin condition represents a combined matrix for clonal culture and should not be described as LN-521 alone. For three-dimensional culture, matrix composition, protein or polymer concentration, and stiffness should be reported instead of a two-dimensional coating density.

6.3 Immune cell cultures (e.g., CAR-T Cells, NK Cells)

SFM are increasingly used for standardized CAR-T and NK-cell manufacture, in which the medium must support expansion without compromising phenotype or antitumor function. In CD19 CAR-T workflows, SFM selection can affect expansion and functional performance (Eberhardt et al., 2023; Muthuvel et al., 2025). For NK cells, activation programs based on IL-12/IL-15/IL-18 priming or membrane-bound IL-21 feeder cells can

promote durable effector responses and metabolic activation (Ni et al., 2012; Romee et al., 2016; Ojo et al., 2019). Nevertheless, cytokine support alone may not prevent glycolytic overactivation, oxidative stress, or TGF-β-mediated suppression.

Metabolic interventions should therefore be treated as product-specific adjuncts rather than as universal SFM components. In CD8+ T-cell models, partial glycolytic restriction with 2-deoxy-D-glucose promoted memory-associated differentiation and antitumor activity (Sukumar et al., 2013), whereas transient AKT inhibition improved the expansion of tumor-reactive lymphocytes with memory-like characteristics (Crompton et al., 2015). In NK cells, TGF-β represses mTOR-dependent activation; TGF-β-pathway blockade or TGF-β-resistant CAR-NK engineering may consequently preserve function under suppressive conditions (Viel et al., 2016; Shin et al., 2025). Vitamin C improved proliferation and effector function in human γδ T cells, providing a model-specific example of redox modulation (Kouakanou et al., 2020). These interventions therefore require product-specific evaluation based on expansion and viability, phenotype-related markers such as CD62L/CCR7 and PD-1/TIM-3, redox or mitochondrial status, and cytotoxicity (Table 4).

Table 4 Representative adjunctive interventions relevant to serum-free T- and NK-cell culture and their reporting considerations

Intervention class	Representative example	Rationale	Report with outcome
Metabolic modulator	Partial glycolytic restriction with 2-DG in CD8+ T-cell models	May favor memory-associated differentiation; not a basal-medium component.	State exposure schedule; assess expansion, CD62L/CCR7, and cytotoxicity (Sukumar et al., 2013)
Pathway inhibitor	Transient AKT inhibition during T-cell expansion	Supports memory-like tumor-reactive lymphocyte phenotypes in model systems.	State inhibitor and timing; assess proliferation, phenotype, and function (Crompton et al., 2015)
Suppressive-signal control	TGF-beta blockade or TGF-beta-resistant CAR-NK design	Addresses TGF-beta-mediated repression of NK-cell mTOR signaling in suppressive settings.	State TGF-beta context; assess mTOR-related status and cytotoxicity (Viel et al., 2016; Shin et al., 2025)
Redox adjunct	Vitamin C in human gamma-delta T-cell culture	Example of context-dependent redox modulation; not a universal T/NK supplement.	State vitamin form/dose; assess ROS, viability, cytokines, and cytotoxicity (Kouakanou et al., 2020)

Note: Examples are experimental adjuncts, not universal basal SFM components. Product-specific optimization should report cell source, activation system, culture medium, intervention timing, dose, and functional endpoints.

6.4 The application of primary cells in tissue engineering

Serum-free media have expanded from traditional primary cell culture to three-dimensional organoids and organ-on-a-chip systems (Panella et al., 2021; Kang et al., 2024). CDM can reduce serum-associated batch variability and, in selected systems, support more reproducible long-term expansion for tis-

sue-engineering and disease-modeling applications (Panella et al., 2021).

Unlike two-dimensional monolayer cultures, organoid and primary-cell models are subject to size-dependent nutrient and oxygen gradients and matrix-dependent diffusion limitations. Medium composition, matrix properties, construct size, and flow, perfusion, or agitation conditions should therefore be optimized as a coupled system (Homan et al., 2019). In addition to size, viability, and lineage- or function-specific markers, model-appropriate metabolic readouts such as OCR and ECAR may be used to evaluate the bioenergetic state of organoids (Ludikhuize et al., 2021). Long-term culture should also assess structural and genomic stability as relevant to the organoid model and its intended application (Huch et al., 2015).

Intestinal organoid technology uses chemically defined formulations with specific small molecules to regulate self-renewal and differentiation. A tunable system incorporating TpC factors (TSA, pVc, CP673451) in serum-free basal medium balances LGR5+ stem cell maintenance with the generation of Paneth cells, goblet cells, and enteroendocrine cells without separate differentiation steps (Yang et al., 2025b). This approach facilitates high-throughput applications and reproducible disease modeling for inflammatory bowel conditions.

In hepatic organoid culture, researchers have established chemically defined, animal-origin-free media to generate iPSC-derived liver buds. Sekine et al. developed chemically defined, animal-origin-free formulations to differentiate hepatic, endothelial, and mesenchymal lineages and then assembled these cell types into all-iPSC liver buds. The resulting liver buds retained hepatic functions, including CYP3A4 and CYP2C9 activity, comparable to those generated using conventional media (Sekine et al., 2020). Separately, Harrison et al. developed a scalable, ECM-independent suspension protocol for generating tissue-like vascularized liver organoids from hPSCs (Harrison et al., 2023). The protocol used CHIR99021 during early mesendoderm induction but subsequently employed KnockOut Serum Replacement and FBS-containing media; therefore, it should not be described as fully chemically defined (Harrison et al., 2023). The resulting organoids contained hepatocytes, cholangiocytes, endothelial populations, hepatic stellate cells, and Kupffer-like macrophages and exhibited vascular luminal structures, CYP1A2 and CYP3A4 activity, and features consistent with hepatocyte polarization and bile-canalicular organization (Harrison et al., 2023). Together, these complementary platforms support clinically oriented defined-medium development and provide experimental models for studies of liver development, drug metabolism, and hepatotoxicity, although their application-specific predictive performance requires further validation (Sekine et al., 2020; Harrison et al., 2023).

Beyond static organoid cultures, SFM has become integral to dynamic microphysiological systems (organ-on-a-chip), where defined formulations improve control over the extracellular environment. Gut–liver microphysiological systems using SFM have revealed potential crosstalk mechanisms regulating drug metabolism and supported quantitative prediction of human pharmacokinetic responses (Herland et al., 2020; Kurniawan et al., 2024; Wang et al., 2024).

For specific primary cell reprogramming, human chemically induced hepatic progenitor cells (hCdHPCs) generated using defined small molecule cocktails (HGF, A-83-01, and CHIR99021) exhibit persistent self-renewal capacity over at least 10 passages, differentiating into functional hepatocyte-like cells expressing albumin and CYP3A4, as well as cholangiocytes, which efficiently engraft in liver injury models (Katsuda et al., 2017; Katsuda et al., 2019). Lineage-specific MSC tissue-engineering studies provide complementary examples. BMP-2 enhanced the TGF-β3-mediated chondrogenesis of human bone-marrow MSCs in alginate beads (Shen et al., 2009). In osteogenic systems, BMP-2-functionalized electrospun silk scaffolds and pre-

mineralized silk-fibroin scaffolds promoted calcium deposition and bone-related marker expression under osteogenic conditions (Li et al., 2006; Kim et al., 2008). For tendon engineering, a defined SFM containing FGF-2, TGF-β3, and IGF-1 acted synergistically with tendon-like substrate topography to promote MSC tenogenesis (Li et al., 2023). More recently, a hypoxic spheroid platform supported serum- and antibiotic-free adipogenic, chondrogenic, and osteogenic differentiation of primary MSCs from different tissue sources, although umbilical-cord-derived MSCs did not achieve osteogenesis (Moldaschl et al., 2024).

6.5 Application of SFM in virus production and vaccine development

Serum-free, suspension-adapted HEK293 cell systems provide scalable platforms for producing virus-like particles and the influenza virus (Petiot et al., 2011; Gashti et al., 2024; Gashti et al., 2025). For SARS-CoV-2 virus-like particle (VLP) vaccine development, HEK293SF-3F6 cells cultured in animal-component-free SFM were used to produce Gag – Spike chimeric VLPs through the co-transient transfection of plasmids encoding the SARS-CoV-2 spike glycoprotein and the HIV-1 Gag structural protein (Gashti et al., 2024). The downstream process combined tangential flow filtration with affinity chromatography to obtain purified VLP preparations (Gashti et al., 2025). In murine immunogenicity studies, these VLPs elicited spike-specific humoral and T-cell-mediated immune responses, with the Quil-A-adjuvanted formulation producing the strongest cell-mediated response among the tested formulations (Gashti et al., 2024). For influenza virus production, HEK293SF cells cultivated in serum- and animal-component-free medium in a 3-L perfusion bioreactor reached a maximum infectious-virus titer of 3.3×10^{11} IVP/mL and a total production of 1.0×10^{15} IVP at three days post-infection; the infectious-particle titer was nearly one order of magnitude higher than that obtained in the batch culture (Petiot et al., 2011). Collectively, these studies support the technical feasibility and scalability of serum-free HEK293 platforms for VLP and influenza-virus production, although product-specific process optimization and regulatory validation remain necessary before clinical manufacturing (Petiot et al., 2011; Gashti et al., 2024; Gashti et al., 2025).

7 Future development trends and research directions

7.1 Intelligent and automated culture medium development platform

Model-informed methods are increasingly being evaluated alongside empirical screening in bioprocess development. Process models can support data interpretation, experimental prioritization, and the development of digital or automated workflows, but their application still requires experimental verification (Narayanan et al., 2020). Hybrid models combine mechanistic assumptions with data-driven components and may support process optimization after model verification and validation (Gaddem et al., 2024). In practical bioprocess applications, Raman spectroscopy has been combined with indirect hard modeling to provide in-line glucose measurements for feedback control in yeast fermentation (Müller et al., 2024). In CHO cell culture, variable-selection strategies improved Raman-based models for the real-time prediction of glucose, lactate, ammonium, and viable cell density, although the study evaluated process monitoring rather than automated medium supplementation (Dong et al., 2024). These capabilities extend rather than replace conventional offline measurements and operator-guided decision-making, as summarized in Fig. 5.

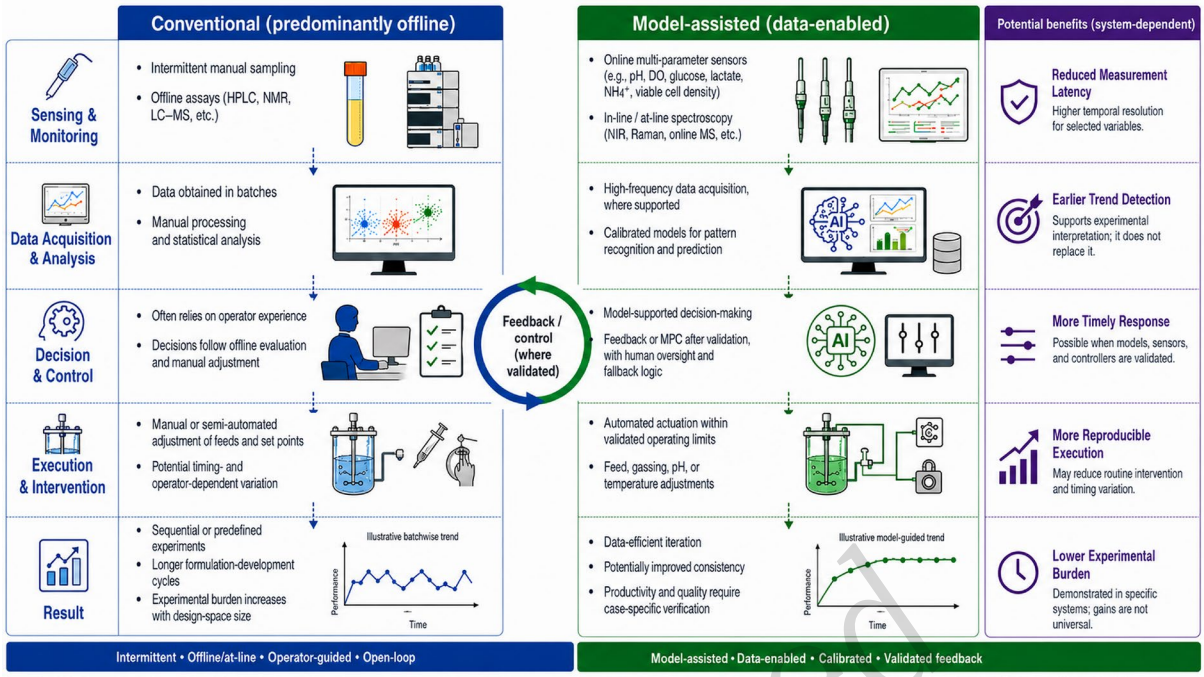

Fig. 5 Conventional and model-assisted workflows for cell-culture process development and operation. This author-generated schematic contrasts predominantly offline, operator-guided workflows with data-enabled, model-assisted workflows across monitoring, data analysis, decision-making, intervention, and evaluation. Model-assisted operation complements rather than replaces reference assays, experimental validation, human oversight, and fallback procedures; its benefits are process-dependent and require model calibration and validation. The schematic was synthesized from representative studies rather than reproduced from a single published source (Dong et al., 2024; Müller et al., 2024; Hashizume and Ying, 2025; Narayanan et al., 2025). Abbreviations: AI, artificial intelligence; DO, dissolved oxygen; HPLC, high-performance liquid chromatography; LC–MS, liquid chromatography–mass spectrometry; MPC, model predictive control; NH_4^+, ammonium ion; NIR, near-infrared spectroscopy; NMR, nuclear magnetic resonance.

Within this framework, in-line or at-line sensing can increase the sampling frequency of selected measurable variables, while calibrated predictive models may support state estimation and earlier trend detection. Nevertheless, offline reference assays remain necessary for model calibration and verification (Dong et al., 2024; Müller et al., 2024). Once sensors, models, and controllers are validated within a defined operating domain, feedback or model predictive control can support timely adjustment of feed rates or other process set points, while retaining human oversight and fallback procedures. Model-assisted experimental design may also reduce the number of experiments required for specific medium-design problems. In one application, Bayesian optimization produced reported reductions in experimental requirements by factors of 3–30 for peripheral blood mononuclear cell (PBMC)-medium optimization and 10–30 for yeast-derived recombinant-protein production, relative to the estimated requirements of standard methods (Narayanan et al., 2025). In another study, biology-aware machine learning evaluated 364 formulations within a 57-component SFM design space, resulting in an approximately 60% increase in CHO-K1 peak viable cell density relative to the commercial media examined (Hashizume and Ying, 2025). Thus, the potential benefits summarized in Fig. 5—including reduced measurement latency, earlier trend detection, more timely response, more reproducible execution, and lower experimental burden—should be interpreted as process and system-dependent outcomes requiring process-specific calibration and prospective experimental confirmation.

7.2 Sustainable and ethical innovation of culture media

Sustainable SFM development is moving beyond replacing animal-derived components toward scalable, environmentally responsible manufacturing. Microbial expression systems can provide animal-origin-free growth factors; Venkatesan et al. produced bioactive FGF2, IGF1, PDGF-BB, and TGF-β1 in *Escherichia coli* for cell-culture applications (Venkatesan et al., 2022). A 2026 review further summarized microbial recombinant-protein manufacturing, including host selection, strain engineering, fermentation optimization, and downstream purification (Chen et al., 2026). A retrospective analysis of products entering clinical development between 1993 and 2023 illustrated the scale of the cell and gene therapy development landscape, identifying 995 cell and gene therapy products corresponding to 1961 development programs. Among these products, regulatory approval had been secured for at least 44 (Elsallab et al., 2025). This broad development pipeline underscores the need for robust and scalable manufacturing systems, although these data should not be interpreted as directly quantifying demand for specific recombinant growth factors or CDM. Regulatory guidance emphasizes the risk-based control of materials used in cell and gene therapy manufacturing. The FDA's 2024 draft guidance recommends characterizing human- and animal-derived materials by source, qualification, adventitious-agent risk, and lot-to-lot variability; recombinant proteins also require appropriate qualification because they may contain expression-system-derived impurities (US FDA, 2024b). European Medicines Agency (EMA) guidance similarly requires the evaluation of culture reagents and biologically active additives for identity, purity, sterility, biological activity, and adventitious agents, while recommending synthetic or defined non-animal-derived alternatives where applicable (EMA, 2008). Nevertheless, environmental sustainability remains incomplete without parallel progress in waste valorization; scalable technologies for recovering, regenerating, and reusing spent media nutrients remain underdeveloped and warrant accelerated research and development investment (Carmona Marques et al., 2025).

7.3 Emerging treatment models and their implications for SFM design

Emerging in vivo and point-of-care cell-manufacturing models are expanding the design requirements for defined culture and cell-support systems. In vivo CAR-T manufacturing exemplifies this shift: implantable biomaterials such as alginate scaffolds or supramolecular hydrogels recruit T cells, enable gene editing, and support expansion within the tumor microenvironment, thereby generating functional CAR-T cells without ex vivo manipulation. The Brudno group's alginate scaffold platform demonstrates this innovation across two generations. The MASTER system generates and releases CAR-T cells in situ within 24 hours, exhibiting enhanced expansion kinetics and greater persistence compared with standard intravenous infusion in lymphoma models (Agarwalla et al., 2022). Its successor, the Drydux scaffold, further refines the process with a streamlined three-day preparation timeline. Notably, preclinical studies have demonstrated that CAR-T cells generated via the Drydux platform exhibit over 150 days of sustained persistence in murine models of systemic lymphoma, with observed tumor regression in ovarian and pancreatic cancer models (Pandit et al., 2024). However, these data are derived from immunodeficient mouse xenograft models, and translation to human patients requires the validation of in vivo transfection efficiency, the control of CAR-T expansion kinetics, and the mitigation of biomaterial-induced foreign body responses (Agarwalla et al., 2022). Other strategies have validated alternative technical pathways: a lymph node–mimetic poly(lactic-co-glycolic acid) (PLGA) scaffold developed by a Chinese research team achieved up to 50-fold in vitro and 15-fold in vivo CAR-T cell expansion (Liao et al., 2024). Meanwhile, Zhu et al. developed an injectable supramolecular hydrogel through

host–guest self-assembly that delivered a CAR-encoding plasmid under a T-cell-specific CD2 promoter, enabling the local in situ generation and accumulation of CAR-T cells at tumor sites in humanized mouse models (Zhu et al., 2024). Collectively, these "in vivo bioreactor" platforms are redefining the conceptual and functional boundaries of SFM. They require the rational design of implantable or injectable biomaterials with exceptional biocompatibility and biofunctionality, thereby expanding the scope of SFM research from classical nutrient supplementation toward active microenvironment engineering.

The emergence of point-of-care (POC) manufacturing has promoted the use of closed, semi-automated cell-processing systems in academic hospitals. In a phase I trial of ARI-0001 for CD19-positive B-cell malignancies, Castellà et al. used the CliniMACS Prodigy system to integrate T-cell selection, lentiviral transduction, and IL-7/IL-15-supported expansion. Of the 28 manufactured products, 27 met all release specifications, supporting the feasibility of clinical-grade CAR-T cell production at an academic medical center (Castella et al., 2020).

Against this evolving landscape, iPSC-derived NK cells provide a renewable and potentially standardized starting material for off-the-shelf cell manufacturing. Lupo et al. established a chemically defined, serum-free, feeder-free process to differentiate functional NK cells from iPSCs (Lupo et al., 2021). For expansion, Kikuchi et al. developed an aerated stirred-bioreactor process that scaled iPSC-derived NK-cell culture from 1 L to 10 L and achieved approximately 1000-fold expansion while maintaining comparable cell quality (Kikuchi et al., 2025b). Realizing standardized allogeneic cell-manufacturing workflows requires coordinated control of iPSC generation, lineage differentiation, and downstream expansion. Elanzew et al. developed the StemCellFactory, a modular automated platform integrating fibroblast expansion, feeder-free Sendai virus-mediated reprogramming, automated colony isolation, and parallel hiPSC clone expansion, producing footprint-free hiPSCs within 3 weeks (Elanzew et al., 2020). This platform demonstrates the feasibility of modular automation for iPSC generation and expansion, while lineage-specific differentiation and large-scale NK-cell expansion require separately optimized media and process modules.

8 Challenges and limitations

The adoption of SFM remains constrained by economic, biological, manufacturing, and regulatory factors. The relative importance of these constraints varies by production platform and intended application.

8.1 Economic and manufacturing barriers

The economic implications of adopting CDM vary substantially among production platforms and operating modes. In cultivated-meat production, media components have been estimated to account for 55%–95% of total production costs (Pajčin et al., 2022), whereas raw materials account for approximately 10%–15% of the cost of goods sold in established monoclonal-antibody manufacturing (Farid, 2007). In intensified mammalian-cell culture, media consumption can become a significant cost driver, but its economic effect depends on the operating mode and achieved productivity. In a CHO monoclonal-antibody comparison, perfusion achieved substantially higher volumetric productivity with a media cost per gram of antibody comparable to that of fed-batch culture, whereas concentrated fed-batch incurred the highest media cost (Xu et al., 2017). Accordingly, the media-related economic burden should be evaluated on a process-specific, unit-product basis

rather than as fixed percentages across manufacturing platforms.

The COVID-19 pandemic exposed vulnerabilities in pharmaceutical and cell-and-gene-therapy supply chains, including raw-material shortages, manufacturing interruptions, and logistical constraints (Cundell et al., 2020; Qiu et al., 2021). One-third of surveyed cell-therapy companies reported manufacturing delays or complete stoppages, while one-fifth reported disruptions in supply procurement (Qiu et al., 2021). Recommended resilience measures include risk-based inventory management, qualifying alternative suppliers, and multisourcing critical materials (Cundell et al., 2020). These findings support supply-chain diversification, although the extent of recovery and its economic effects require time- and sector-specific evaluation.

8.2 Biological adaptability issues

Adaptation to serum-free conditions is cell- and process-dependent. Industrial CHO platforms have a long history of serum-free and chemically defined medium development (Ritacco et al., 2018), whereas primary MSC responses depend strongly on medium formulation and production protocol. In Wharton's jelly-derived MSCs, a validated xeno- and serum-free system supported clinical-scale expansion while maintaining phenotype, multilineage differentiation, genomic stability, and immunosuppressive activity (Swamynathan et al., 2014). A recent comparison of customized media for umbilical-cord MSCs further demonstrated formulation-dependent differences in proliferation, senescence, differentiation, and paracrine profiles; selected serum-free formulations improved long-term expansion and stability (Zhao et al., 2024). In CHO cells, stepwise adaptation to serum-free conditions also produced stage-dependent changes in monoclonal-antibody glycosylation, highlighting the need to monitor product quality throughout adaptation (Costa et al., 2013).

8.3 Scalability and regulatory hurdles

At the bioreactor scale, hydrodynamic stress and foam-control strategy are distinct process risks that require separate evaluation. In CHO fed-batch cultures, average shear stress was correlated with decreased antibody titer, and shear sensitivity varied among cell lines, indicating that hydrodynamic characterization should be incorporated into scale-up (Kenmoku et al., 2025). Separately, medium and antifoam selection affected CHO cell growth, aggregation, IgG1 production, and foam control in a micro-bioreactor screening system (Velugula-Yellela et al., 2018). These effects are process- and formulation-dependent and should not be generalized as universal consequences of SFM. Complex CDM with 50–100 components creates a substantial vendor qualification burden and extensive good manufacturing practice documentation requirements (Combe and Sokolenko, 2021). Regulatory expectations for media components and other raw materials should not be framed as a simple FDA–EMA dichotomy. Both regions use risk-based approaches to material characterization and control EMA, 2008; US FDA, 2024b), although product classification, submission pathways, and documentation requirements differ between the United States and the European Union (Iglesias-López et al., 2019). China has likewise operated under a dual-track regulatory framework for cell therapy since 2017, alongside rapid growth in clinical research and product development (Du et al., 2024). More broadly, country-specific requirements were among the most frequently reported challenges in a survey of European ATMP developers (Ten Ham et al., 2018).

8.4 Knowledge gaps

Predictive metabolomics and machine-learning-assisted medium design remain developing approaches, and clone-specific experimental validation is still required (Hashizume and Ying, 2025; Narayanan et al., 2025). Media recycling also remains under investigation. In an ambr® 250 perfusion model for monoclonal antibody production, replacing 25%–50% of fresh medium with recycled Protein A flow-through reduced cumulative productivity by 13%–30% while maintaining product quality; process mass intensity calculations nevertheless indicated improved material efficiency through lower water consumption (Madabhushi et al., 2022). Because these results were obtained under a specific perfusion configuration, the numerical ranges should not be generalized across cell lines, media formulations, or manufacturing platforms. More recent work used ion concentration polarization to remove inhibitory ionic waste products from spent perfusion medium, allowing the regeneration and reuse of up to 75% of the medium without reducing cell viability and projecting up to a 33% improvement in water process mass intensity (Wynne et al., 2025).

9 Conclusions

This review reframes SFM development as a metabolism-guided design problem rather than a linear serum-replacement process. The central principle is to select, dose, and dynamically adjust medium components according to cell-specific metabolic constraints and process endpoints. For industrial cell lines, the dominant challenge is often metabolic burden and product-quality control; for stem cells, maintaining identity requires coordinated regulation of soluble factors and matrix signals; for immune cells, expansion must be balanced against exhaustion, oxidative stress, and functional potency. Future SFM development will therefore depend on integrating metabolomics, metabolic modeling, high-throughput screening, and real-time process monitoring into closed-loop optimization strategies (Schinn et al., 2021; Eberhardt et al., 2023; Hashizume and Ying, 2025; Narayanan et al., 2025).

Animal-component-free and chemically defined formulations can reduce xenogeneic risk and improve consistency, but their adoption remains subject to product- and process-specific, risk-based regulatory evaluation (EMA, 2008; US FDA, 2024b). Emerging AI-assisted and medium-recycling strategies may improve development efficiency and sustainability (Narayanan et al., 2020; Gaddem et al., 2024; Carmona Marques et al., 2025; Wynne et al., 2025), although clone-specific metabolic requirements continue to preclude the development of universal formulations (Combe and Sokolenko, 2021; Hashizume and Ying, 2025).

In summary, SFM development requires coordinated control of medium composition, cellular metabolism, process operation, and product-quality endpoints. Further progress will depend on experimentally validated integration of metabolic measurements, automated monitoring, and cell-type-specific formulation strategies across biologics and cell-therapy manufacturing.

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Author contributions

Xuan TAN conducted the literature search, collected and analyzed the relevant data, and drafted and revised the manuscript.

Qubo ZHU contributed to the study conception and design, data analysis and interpretation, and manuscript writing and editing, and provided supervision and funding acquisition. Both authors have read and approved the final manuscript.

Compliance with ethics guidelines

Xuan TAN and Qubo ZHU declare that they have no conflicts of interest. This review does not involve any studies with human participants or animals conducted by either of the authors; therefore, ethical approval and informed consent were not required.

Declaration on the use of generative AI tools

During the preparation of this manuscript, the authors used Kimi (Moonshot AI) to assist with literature organization and subsequently used ChatGPT (OpenAI) for language polishing and formatting checks. All AI-assisted outputs were reviewed and revised by the authors, who take full responsibility for the final content of the manuscript.

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