Review

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Review

Forging a Xeno-Free Future for Cell-Culture Based Vaccines and Biopharmaceuticals

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Abstract

Cell lines represent a cornerstone of modern vaccinology, gene therapy, and biopharmaceutical production, serving as platforms for viral propagation, recombinant protein expression, and vector development. Classical lines such as Vero, MDCK, and HEK293 have provided robust systems for vaccine manufacturing, while more recently developed designer lines, including PER.C6, EB66, and HEK clones, offer enhanced yields and defined characteristics suited to regulatory and industrial requirements. Advances in cell engineering, ranging from spontaneous adaptation to suspension culture to targeted genetic modification, have enabled improved scalability, stability, and biosafety, addressing the increasing demand for global vaccine and gene therapy supply. Natural and induced genetic changes, including immortalization strategies, glycoengineering, and viral receptor modulation, have further expanded the versatility of production hosts. Alongside these developments, a critical shift is underway from serum-dependent media to serum-free, chemically defined, and xeno-free alternatives, which minimize variability, reduce contamination risks, and align with Good Manufacturing Practice standards. This review synthesizes the evolution of cell lines in vaccine and gene therapy applications, examines methods of engineering and optimization, and evaluates prospects for serum-free and xeno-free platforms. By integrating historical context with contemporary advances, it highlights both the opportunities and caveats that shape the trajectory of next-generation biomanufacturing.

Keywords: cell culture; serum-free media; virology; vaccinology; biotherapeutics

1. Introduction

The practice of variolation, which involved the deliberate inoculation of material from smallpox lesions into healthy individuals to induce a milder form of the disease and confer immunity, was an early method employed in the fight against smallpox. [35] Despite its risks, it remained the only available form of immunisation until Edward Jenner's observations in the late 18th century. Jenner noted that milkmaids rarely contracted smallpox and, in 1796, inoculated an eight-year-old boy, James Phipps, with material from a cowpox pustule. The procedure demonstrated that cowpox infection could elicit a mild immune response and confer cross-protection against smallpox and related orthopoxviruses, paving the way for vaccination- a term derived from the Latin word vacca, meaning cow.[78] and with that, immunization faced its first paradigm shift.

Vaccination as we know it, is a form of active immunity whereby a controlled dose of a pathogen and/or its pathogenic subunit, a spike protein of the COVID19 virion per se, is used to trigger a complete immune response by "priming" host defense cells to specific stimuli.[74] Vaccines are designed to achieve pathogen-specific antibody memory by alerting the Innate Immune system prior to exposure by presenting antigens to Antigen Presenting Cells which consequently trigger MHC class I and II along with CD8+ & CD4+ T cell activity respectively, thereby assuring pathogen-specific adaptive immunity.[10]

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Various vaccines have employed similar rationale to combat pathogens, but their production methods differ. Vaccines developed for bacteria have a stand-alone bioprocess whereby the production of bacterial vaccines generally focus on the direct multiplication of the micro-organism. In the right media and environment, bacteria can carry out all metabolic and cellular functions necessary for sustenance.[79] This simplicity allows scientists to readily isolate and cultivate bacteria, enabling the production of vaccines using different approaches, including killed whole-cell vaccines, acellular vaccines, and subunit vaccines, as exemplified by vaccines against Salmonella Typhi, Haemophilus influenzae type B, Vibrio cholerae and Bordella pertussis.[23]

Viral pathogens, unlike their bacterial counterparts, cannot survive without a host. Due to their nature of being obligatory intracellular parasites, viruses lack the cellular machinery necessary for independent replication.[65] This fundamental dependency and truth of life extends into viral vaccinology and virology at large that without access to a suitable host, viruses remain dormant and incapable of propagation.[20]

The table below highlights the importance of viruses in the field of biology more than just vaccinology and therefore, implied significance of virus-friendly hosts as expression platforms.

Table 1. Importance of mammalian-host systems for various therapeutic virology-based bioproduction platforms.

Application	Virus/ Vector of choice	Host system	Importance of mammalian-host system	References
Vaccinology	IFv, DengV, NoV, Sars-Cov2 and potentially any virus that has been identified/sequenced	Mammalian cell lines CEF MDCK HEK293 / 293F MRC5 Vero	Viral virulence decreases with each cell passage thereby obtaining inactive/live attenuated vaccines. Certain mammalian cell lines are preferred as they perform appropriate PTM's and protein folding which gives rise to Subunit and Epitope based vaccines.	S. Furkan Demirden et al., 2024
Gene therapy	AAV, ADV, Lenti/Retrovirus based on desired length of gene expression (transient/stable expression)	HEK293 / 293T MDCK BHK21	HEK293 (T) cells are favored for ADV and AAV vector production due to their efficient transfection and consistent viral titre yield FDA approved AAV-RPE65 gene therapy using HEK293 cell line to treat inherited retinal dystrophy [Luxturna®] since 2017	Bulcha et al., 2021
CAR-T Cell therapy	HIV-1 based Lentivirus and γ-Retroviral vectors	HEK293 / 293T / 293F	Viral plasmids are transfected into the host cell for assembly into viral vectors which is then used to genetically modify T cells in CAR-T therapy. HEK293T cell lines, expressing SV40 large T antigen, boost viral vector titres by enhancing replication of transfected plasmids containing the SV40 origin of replication [Ex. FDA approved Kymriah® using HEK293 assembled LV for CD19 directed CAR-T cell therapy]	Tan et al., 2021 Na Kyung Lee & Jong Wook Chang, 2024
Oncolytic therapy	MG1-derived Maraba virus, Recombinant Poliovirus (PVSRIPO), Augmented HSV-1, VSV-NDV	EB66 suspension quail cells Vero BHK21 HEK293 A549 lung carcinoma	Vero cells are interferon-deficient, making them highly susceptible to infection due to defective antiviral defenses. Culturing oncolytic viruses like ADV and HSV require mammalian cell lines to	Max Planck Institute Sven Göbel et al., 2022

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		epithelial cell line	retain viral tropism, infective and viral potency. A549 lung adenocarcinoma cell line is used to assess and screen cytolytic activity of oncolytic viruses and serves as a platform to study host-pathogen interactions i.e., COVID19, IfV.	

Above all, a perfect host must be alive—capable of providing the metabolic infrastructure that viruses rely on for their replication.[10] Notably, viruses can be cultivated in vivo or in vitro. While it was just in the recent past when cattle and embryonated bird eggs were used to perpetuate viral matter, the downsides of in vivo propagation were revealed soon over time and forced the scientific community to seek solace in cell lines and microorganisms as alternative hosts.[79] Moreover, the utility of viruses extends to beyond production platforms for vaccines but also as indispensable tools for viral vector gene-therapies and protein expression. Table 1.0.1 highlights the biological relevance of key viruses in the field of biology and implied significance of virus-compatible hosts as expression platforms.

This paper will examine the indispensable role of cell culture and its fundamental components in advancing the field of virology. It will underscore the pivotal importance of cell culture in vaccinology, trace notable trends in cell culture–based bioproduction and explore modern adaptations within the discipline. Furthermore, it will discuss the concept of "xeno-free" and "animal component–free" cell cultures, defining these terms and elucidating the implications of eliminating animal-derived substances in the production of biologics using cell culture systems.

2. Cell Cultures & Their History

The art of cell culture is a versatile technique whereby prokaryotic or eukaryotic cells are removed from the host and cultivated in a conducive environment, for functional and translational research.[83]

While multiple factors influence cellular productivity and viability, cell culture media composition and in vivo supplementation remain pivotal in recreating a "cellular safespace" which is a biomimetic microenvironment, like that of its host, for optimal cell performance.[99,100]

Culture media are broadly classified as natural or synthetic. Natural media include biologically derived fluids such as blood, cerebrospinal fluid, and interstitial fluids, whereas synthetic basal media such as Dulbecco's Modified Eagle Medium (DMEM), Eagle's Minimum Essential Medium (EMEM), and RPMI-1640 were developed to support cell maintenance in research and industrial applications.[67]

Synthetic media are typically formulated without certain labile cofactors and proteins required for sustained proliferation, thereby extending shelf-life and stability, since supplemented media degrade rapidly.[1,95] Beyond that, the notion of "one-fits-all" does not apply to culture media because each cell and line of work has its own metabolic and physiological needs that must be supplemented accordingly.[95]

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A breakthrough occurred in the late 1950s, when Theodore Puck introduced fetal bovine serum (FBS) as a supplement to sustain fastidious cellular growth. Since then, FBS has become synonymous with serum-dependent media across laboratories worldwide.[76] Despite extensive metabonomic and proteomic analyses, its composition remains incompletely defined.[85] However, FBS is known to be enriched in cytokines, growth factors, amino acids, and fat-soluble vitamins, while containing relatively low levels of γ-globulins which are known to exert growth-inhibitory effects.[48] This unique biochemical balance underpins the effectiveness of FBS in supporting difficult-to-culture cell lines and explains its widespread use at 2–10% (v/v) in serum-dependent systems. [95]

The utility of cultured cells was quickly realized in vaccinology. Early vaccine production employed primary human fetal cell strains such as Wistar Institute-38 (WI-38) and Medical Research Council-5 (MRC-5) lung fibroblasts to generate live-attenuated and inactivated vaccines against rubella, poliovirus, hepatitis A, rabies, and varicella.[24] While safe and rigorously tested, these strains had finite proliferative capacity and required meticulous handling, limiting their scalability and long-term use.[75]

It was only during the late 20th century when Vero cells, derived from the kidney epithelial cells of the African green monkey (Chlorocebus sabaeus), were established as the first continuous cell line approved by the World Health Organization (WHO) for the manufacture of human viral vaccines, owing to their broad permissiveness to a wide range of viruses.[79] Since their landmark approval for inactivated poliovirus vaccine (IPV) production, Vero cells have remained a regulatory-accepted workhorse for large-scale vaccine manufacture.[24] More recently, they were pivotal in the global COVID-19 response, with sublines such as Vero E6 enabling the efficient propagation of SARS-CoV-2 for the production of inactivated vaccines like CoronaVac, where they supported higher viral titres compared to other cellular hosts.[79]

The escalating global population, coupled with rising immunization demands and the emergence of novel infectious diseases, has spurred a critical need for higher-throughput vaccine production, driving the proportional growth of cell-culture based vaccinology.[51,100]

2.2. Primary Cell Cultures, Cell Strains, Continuous Cell Lines

A tissue sample is used to obtain the primary cell culture- the best ex vivo representation of cells as they are derived straight from the tissue of an organism or human [101] usually via FNAC, liquid biopsies, pleural effusion, resections or autopsies.[42] They retain the same physiology and properties of host tissue, including cellular heterogeneity which can be very beneficial for single cell RNA sequencing, oncology and transcriptomic studies, but due to their finite lifespan, primary cell cultures are not the best choice for virus expression platforms.[77]

This limited replicative capacity is referred to as the Hayflick limit, which dictates the finite number of divisions a cell population can undergo.[32] Normal or finite cells and their cell lines, have a natural limit on how many times they can divide, and with each cell division, telomeres present on chromosomal ends become shorter, before cells lose their ability to divide ever again. This is known as senescence.[19]

Primary cultures require cells from tissue samples to be cultured in their log phase which is right before confluency- the stage at which cells exhaust all available substrate and space. Once this is achieved, cells must be sub-cultured or passaged which involves replenishing culture media and discarding cells via trypsinization to prompt healthy cell growth along with replenishing culture.[31]

From a primary cell culture, a cell line is achieved upon the first subculture. Further refinement of a cell strain can be achieved from this by sub-populating a select population/group of cells such as the WI38 and MRC5 fibroblast cell lines, which albeit served as substrates for various viral vaccines, are subject to the Hayflick limit. [27]

Furthermore, despite their invaluable role as expression platforms for a wide array of critical vaccines, the WI38 and MRC5 cell lines originated from elective abortions. This derivation, irrespective of the profound public health benefits achieved, was sufficient to ignite ethical objections

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from certain segments of the public, thereby driving the demand for alternative cell lines that could circumvent such moral sensitivities. [75]

Today, we have an array of continuous cell lines, spanning the animal, avian, human and insect kingdoms. Continuous cell lines were developed to eliminate the disadvantages of finite cell lines and diploid cell strains which were limited by senescence.[56] As the name suggests, continuous cell lines are able to override the natural calling of senescence and proliferate continuously in vitro, thereby earning the designation of "Immortal" cell lines.[56] Cells are able to do so by means of natural and spontaneous genome transformation or by inducing genetic manipulation via chemical and viral vectors. [79]

The first immortal cell line ever cultivated and used in biologics was the HeLa cell line. These cervical adenocarcinoma cells were obtained from 31-year-old Henrietta Lacks in 1951 and found to exhibit an immortal phenotype. This was primarily due to a human papillomavirus type 18 (HPV-18) infection, which integrated viral oncogenes E6 and E7 into the host genome.[54] As a result, the E6/E7 proteins caused sustained inactivation of the tumour suppressor gene p53. In parallel, there was overexpression of human telomerase reverse transcriptase (hTERT) and the telomerase RNA component (hTR), leading to maintained telomere length.[97] Together, these factors allowed HeLa cells to bypass senescence, making them cancerous and "immortal", providing a foundational tool for modern biomedical research.

Other continuous cell lines such as Vero cells, Madin Darby Canine Kidney (MDCK)-derived from the primary culture of the kidney of an adult female Cocker Spaniel dog and Chinese Hamster Ovary (CHO) cells, are some examples of cell lines that were subject to natural transformation which resulted in their mutant immortal phenotype.[99]

In contrast, the HEK293 (Human Embryonic Kidney 293) cell line originated from a primary culture of kidney cells from a female fetus and was genetically engineered to attain immortality through the integration of adenovirus type 5 (AdV5) DNA.7

In 1973, Frank Graham set out to investigate why certain adenoviruses caused cancer by transfecting primary human embryonic kidney cells with fragments of the human adenovirus type 5 (AdV5) genome using calcium phosphate precipitation, a stable transfection method that enables foreign DNA to integrate into the host genome.[41] Unexpectedly, the viral oncogenes E1A and E1B stably integrated, driving continuous proliferation by forcing quiescent cells into the cell cycle and bypassing apoptotic checkpoints, which ultimately led to the establishment of a continuously proliferating HEK293 cell line.[30]

2.3. Types of Cell Culture Techniques, Cell Lines & Designer Cells

The selection of an appropriate expression platform to be used for bioproduction is arguably the most important stage of the entire production process because it is the functional, genetic and metabolic characteristics of each cell line that determines suitability for diverse biotechnological settings.[66]

For instance, cells cultured in vitro fall under one of two categories based on their growth characteristics and morphology: Adherent and Suspension cell cultures. A brief description of both culture environments are listed in Table 2.3.1. In terms of serving as expression platforms for vaccinology, mAB production, recombinant protein expression and other modalities, both anchorage dependent and independent cell lines have been used.[22,103]

Adherent cultures have become ubiquitous with a list of bio-modalities spanning as expression platforms for viral propagation, cell and gene therapies and immuno-oncology with a multitude of applications.[4] For instance, mammalian lines such as CHO and HEK293 are preferred for recombinant protein expression, with HEK293 often delivering human-like post-translational modifications (PTM's) and faster transgene expression, while CHO has been utilised as a substrate for Hep. B vaccines as it offers genomic stability and superior up-scalability.[40]

Large-scale bioprocesses such as vaccine manufacturing often fail to achieve the cell densities required when relying solely on adherent culture systems, as these are inherently constrained by

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surface area and therefore limited in scalability.[4] Consequently, these limitations have accelerated the adoption of suspension cultures, which offer scalable and cost-effective platforms for industrial bioproduction.[103]

Unlike adherent systems, suspension cultures are not restricted by surface area, allowing cells to proliferate freely within the medium.[11, 103] This property facilitates higher culture densities, which in turn enhances viral titres for vaccine production and increases recombinant protein yields, positioning suspension platforms as the preferred choice for industrial-scale manufacturing. Nevertheless, a major challenge lies in the fact that many cell lines are not naturally suspension-competent and require extensive adaptation or modification to thrive in such conditions.[21]

Fortunately, advances in cell culture technology have enabled the transition of adherent cells into anchorage-independent environments through a process known as suspension acclimation.[84] This involves serial adaptation to reduce attachment and adherent characteristics via cell passaging which introduces selective pressure, thereby promoting the emergence of robust clones of the original culture capable of thriving under "new" conditions and expressing the suspension phenotype.[47]

As an intermediate strategy for scaling up, support materials like microcarriers are used to facilitate cell growth during suspension acclimation of adherent cells.4 Microcarriers are a synthetic or natural bead-matrix that act as a transitional platform by providing cells with a 3D growth surface, thus allowing cells to be cultivated under suspension conditions.[87]

Despite their widespread use, microcarriers have not provided an ideal solution for large-scale adherent culture. Limitations such as shear stress induced by bioreactor agitation, suboptimal cell distribution, challenges in downstream purification, and inconsistent nutrient and oxygen transfer have been reported.[36] These drawbacks highlighted the need for more effective alternatives to achieve both scalability and yield in bioprocessing.

Advances in cell engineering have subsequently enabled the development of "designer" cell lines tailored for specific bioprocesses. Such lines are engineered to enhance productivity, maintain product quality, and align with regulatory standards for biologics manufacturing.[28] Genetic manipulation strategies, including targeted genome editing with CRISPR-Cas9, have facilitated the selection and introduction of desired traits, ultimately generating cell substrates optimized for defined industrial applications.[58]

For instance, genome sequencing of Vero cells found a 9Mb deletion on Chromosome 12 resulting in the complete loss of the Type-1-Interferon gene cluster (IFN-1).[77] This inability to produce certain anti-viral cytokines like IFN-1 allows viruses to replicate without being inhibited by the cell's immune response thereby making it a flagship expression platform for viral propagation. However, Vero cells and their strict adherent nature posed a problem. It was only in 2009 when Paillet et al. generated an offspring cell line, sVero, suitable for growth in suspension and serum free environment which was used for optimized Influenza H1N1 vaccine production. [71]

Another good example of designer cells is the HEK293 cell lineage. It is a long renowned cell line for its high transfectivity, rapid growth rate, and superior post-translational modification (PTM) capabilities in comparison to many other mammalian cell lines.[89]

HEK293 has been used as a platform in generating Chimeric Antigen Receptor T-cells (CAR-T), viral vectors, recombinant proteins, and vaccines. Their efficiency as a host for recombinant adenovirus production is closely linked to their innate susceptibility to viral infection, which is in turn, heavily reliant on the presence of $\alpha v\beta 1$ integrin, functioning as a primary co-receptor to facilitate viral entry. This reliance on $\alpha v\beta 1$ integrin for viral entry in HEK293 cells was notably substantiated by a 2001 study which revealed that experimentally blocking $\alpha v\beta 1$ integrin with a combination of anti-αv and anti-$\beta 1$ antibodies resulted in a substantial 76% reduction in adenovirus internalization.[52]

Subsequent derivatives like HEK293E and 293T were engineered to express specific antigens that enhance episomal vector amplification89, thereby improving protein yield during transient transfection. In contrast, suspension-adapted derivatives like 293F emerged through selective pressure and clonal expansion, favoring variants capable of robust growth in suspension culture.[89]

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While the exact genetic determinants of these adaptations remain incompletely understood, omics studies have shown that HEK293 has diverged significantly from its parental origin, acquiring a transcriptional and metabolic landscape highly optimized for recombinant protein expression and scalable industrial bioprocessing. [82]

Table 2. Key differences between adherent and suspension cell culture systems.

Parameters	Culture type		References
	Adherent	Suspension (Anchorage independent)	
Definition	Anchorage dependent cells that require a solid surface to grow as a *monolayer*	Cultivation of cells does not require attachment to a surface as cells float and proliferate freely.	Weiskirchen et al., 2023
Equipment required	T-flasks, microwell plates, roller bottles	Bioreactors, Erlenmeyer shaker flasks, wave bags	S. Furkan Demirden et al., 2024
Rate limiting steps	Surface area for cell growth is often a rate limiting step because over confluence will increase intercellular competition for media and growth factors. Cell detachment or inability to attach to culture flasks will hinder cell growth.	Concentration of cells within the media of culture vessels creates rate-limiting steps. Therefore, it is essential to monitor the growth rates in suspension cultures over time Shear stress in stirred systems like bioreactors, will lead to cell death if not optimised for.	Segeritz & Vallier, 2017
Up-stream Scalability	Poor scalability due to limited growth areas which make for good cellular study models to observe cell-to-cell adhesions and polarity.	Scalable for large batch, high-density cultures which is necessary in fields like recombinant proteins production and viral vaccinology.	Durocher, 2002
Applications	Inactivated Influenza vaccine [Flucelvax®] made using MDCK cell line as substrate.	Glutamine synthetase-KO CHO cell line used to cultivate recombinant mAB therapies like Rituximab.	Mellahi et al., 2019 Bart et al., 2016
Examples	HEK293, Vero, MDCK-A, MRC5	HEK293-F, EB66, SF9/21, MDCK-S	

Table 2. 3.2: Commonly used designer lines, methods to bring about natural or induced genetic changes, and real-world applications.

CELL LINE	GROWTH REQUIREMENTS	ENGINEERING STRATEGIES	APPLICATIONS	REFERENCES
HEK293T	Adherent/Suspension adapted	Stable transfection of HEK293 with a plasmid encoding SV40 T antigen.	Utilized in suspension culture for high titer rAAV production.	Cell Culture Dish, 2017
HEK293F	Suspension	Subclones of suspension-adapted HEK293 cells were isolated and cloned.	Used for large-scale production of recombinant proteins like rFVIII (NUWIQ®)	S. Furkan Demirden et al., 2024
HEK293E	Suspension	Stable transfection with plasmid encoding viral EBNA1.	Widely used in transgene expressions due to EBNA1 which enhances cell's ability for episomal replication of oriP-harboring plasmids.	Tan et al., 2021
HEK293S	Suspension adapted.	Serial passages in modified MEM.	Glycoengineered variants devoid of *N*-acetylglucosaminyltransferase I (KO-GnTI$^-$) are used for high-throughput production of deglycosylatable glycoproteins, excellent for crystallography.	Chang et al., 2007
Sf9/Sf21	Adapted to adherent and non-adherent conditions, do not require CO2 supplementation and can thrive in serum-free media.	Spodoptera frugiperda 21 was the original line isolated, while their clonal derivative Sf9 was selected for superior suspension growth and stability.	Extensively used in the Baculovirus Expression Vector System (BEVS) for high-yield production of recombinant proteins, virus-like particles (VLPs), and vaccines (Cervarix®). FluBlok® ,recombinant hemagglutinin influenza vaccine produced in *expres*SF+	Michał Sułek & Agnieszka Szuster-Ciesielska, 2025

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PER.C6®	Human embryonic retinal cells, suspension-based	Created by transfecting retinal cells with a plasmid encoding only E1A and E1B, flanked by known sequences, under a CMV promoter.	PER.C6® used as a production platform for Janssen's Ad26.COV2. S recombinant vaccine.	S. Furkan Demirden et al., 2024
CHO-S	Chinese Hamster Ovary (suspension)	Long-term adaptation to serum-free suspension	Gold standard for mAB production, Fc-fusion proteins and therapeutic clotting factors due to superior genetic stability and ability to perform human-like PTM's.	Dumont et al., 2015
EB66®	Duck embryonic stem cells, suspension-based	Proprietary cell line developed by Valvena. Relied on the effects of natural selection and clonal isolation rather than direct genetic manipulation.	EB66® is a highly permissive platform for MVA-based vectors, sustaining robust transgene expression, superior viral titre compared to CEF, rapid scalability (100Lof *rMVA* in 3 weeks).	Léon et al., 2016

[SV40-Simian Virus 40; EBNA1-Epstein Barr Virus Nuclear Antigen 1; oriP- Origin of replication; eSC's- embryonic stem cells; rFVIII- recombinant factor 8; DTMUV- Duck Tembusu Virus; MEM-Minimal Eagles Media; mAb- monoclonal Antibody; CMV- cytomegalovirus; PTM- post-translational modifications; MVA- modified vaccinia virus].

2.4. Cellular and Genetic Engineering

In modern biotechnology, researchers can choose from a diverse array of expression platforms, but selecting the right host is crucial to ensure that the resulting products are human-compatible in their structure and immunological properties. [81,104]

E.coli was prized for its low-cost, protein yield and scalability in stirred tanks, it lacked the fundamental machinery needed for eukaryotic PTM's, rendering it ineffective as a host to produce recombinant proteins or immunologically relevant vaccine antigens.[81] Similarly, eukaryotic systems like Saccharomyces cerevisiae and Pichia pastoris are unable to mimic mammalian PTM's because yeast N-glycosylation is rich in non-homogeneous hypermannosyl structures, which is different from the more complex mammalian glycan structures and can lead to antigenicity.[14,81]

Enter designer mammalian expression systems, which have become the cornerstone of cell culture–based therapeutics, owing to their unparalleled ability to perform human-like PTMs such as glycosylation and sialylation.[55] For certain biologics, these PTMs are absolutely indispensable: mAb's rely on precise Fc glycosylation to engage immune effector functions, clotting factors such as Factor VIII require correct glycosylation for stability[43] and therapeutic efficacy whilst viral glycoproteins such as the SARS-CoV-2 spike protein depend on authentic glycan shielding to preserve their native conformation and antigenicity.[92]

For instance, CHO and HEK293 remain the workhorses of recombinant protein and viral antigen production, but they cannot always reproduce the exact human PTM landscape. This opportunity gave rise to the concept and reality of glycoengineered CHO and HEK cell lines. For instance, mogamulizumab, was the first-ever defucosylated, glycoengineered mAb for the treatment for CCR4-positive T cell leukaemia or lymphoma and its biofactory was the CHO FUT8-/- [fucosyltransferase knockout] cell line.[16]

In vaccinology, scalability is a key consideration when selecting a host system. Although conventional suspension adaptation has yielded several suspension cell lines, the process is time-consuming and can result in reduced growth rates and sensitivity to shear stress in stirred cultures.[4]

Many adhesion molecules mediate interactions between adherent cells and the extracellular matrix (ECM), enabling anchorage-dependent growth. A study titled Comparison of Vero and MDCK cell lines transfected with human SIAT7E gene for conversion to suspension culture demonstrated that transfection with human SIAT7E successfully converted adherent Vero and MDCK cells into suspension cultures.[62]

Located on chromosome 1, the human SIAT7E gene encodes for the SIAT7E enzyme which predominantly catalyzes the biosynthesis of ganglioside GD1alpha from GM1b in the brain which modulates neuronal communication.[13] With the use of differential gene expression analysis, the

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SIAT7E gene was found to be one of the genes that has profound influence on cell adhesion cellular interactions due to its intrinsic sialyltransferase activity.[34]

This study hypothesized that upregulating the transcription of SIAT7E gene conferred a reduced degree of cell adhesion.[62] Experimental validation, performed on MDCK and Vero cells, clarified this phenomenon: cell lines overexpressing SIAT7E, developed a pronounced negative charge on cell surface in comparison to parental cells.[62] Heightened negative charge subsequently led to increased electrostatic repulsion between cells, consequently facilitating their ability to grow in suspension.[12]

Altogether, the trajectory of cell engineering has moved beyond single-trait optimization to the creation of hosts that combine human-like glycosylation, suspension growth, and serum-free adaptability, thereby overcoming previous limitations and reducing the risk of aberrant or immunogenic bioproducts.[80] By replacing and eliminating the need for undefined animal components in culture media, these engineered systems align with Good Manufacturing Practice (GMP) standards and address the increasingly strict regulatory requirements for safety, consistency, and scalability in biomanufacturing.[11]

2.5. Transitioning Towards Xeno-Free Alternatives

Building upon the principles of Good Manufacturing Practices and the clear advantages of traceable, well-defined components in biologics, cell biologists are keenly exploring the replacement of animal-derived substances in cell culture media and its supplements.

Fetal Bovine Serum (FBS) and other animal-derived sera play a pivotal role in cell media by supplementing a rich, albeit undefined, cocktail of essential nutrients, growth factors, hormones, and attachment proteins when supplemented into basal media like EMEM, DMEM or William's Medium E. Functionally, sera aids cell growth, metabolism, and functions as a protective barrier from proteolytic enzymes, thereby preserving cell integrity during routine culture manipulations. [1]

As early as the 1970s, the undefined nature and variability of animal sera had already been recognized as major limitations in reproducible bioprocessing.[98] Over subsequent decades, concerns regarding zoonotic contamination, adventitious viruses, and ethical issues surrounding bovine and porcine-derived components led governing authorities such as the WHO,FDA, EMA, ICH, and national regulatory agencies to mandate rigorous screening of serum-containing media for adventitious agents, while simultaneously encouraging the adoption of xeno-free culture systems.[3] This dual approach reflects both the need to ensure immediate biosafety in existing processes and the long-term goal of fully transitioning to defined, GMP-compliant substrates for human therapeutics.[11]

In addition to that, protein concentration found in media supplemented with 10% sera has a range of 6,200-10,000 mg/L total protein, whilst the concentration for a defined recombinant protein produced in mammalian cells does not usually exceed 1,000 mg/L.[94] This discrepancy poses major challenges in the production of protein-based drugs and recombinant proteins, making serum proteins a huge contaminant, particularly when proteins are structurally or functionally relevant with sera. [94]

In the 21st century, serum-free and chemically defined media emerged as regulatory-preferred platforms for GMP-compliant and xeno-free cell culture. [94] While widespread adoption remains limited by technical and economic constraints, these media are increasingly viewed as the benchmark for safe, consistent, and scalable bioprocessing.[80]

Cell line engineering has advanced in parallel with serum-free media, with established workhorses such as Vero and HEK293 variants now thriving under serum-independent conditions.[8]

For instance, research by Park et al. highlights the potential of marine microalgae extracts as effective alternatives to serum supplementation in cell culture. Specifically, extracts from Dunaliella salina (DS) and Spirulina platensis (SP) significantly enhanced proliferation in MDCK and Vero cells, with DS achieving proliferation rates of 149.56% and 195.50% compared to conventional serum-free media.[72] In addition to supporting higher cell viability, these extracts also increased superoxide dismutase activity, providing antioxidative protection that reduces oxidative stress within cultured cells. [72] By demonstrating that phytochemical-based supplements from microalgae can

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simultaneously promote growth and protect against oxidative damage, this study strengthens the case for reducing reliance FBS.

Furthermore, a product study conducted by MP Biomedicals evaluated the proliferation dynamics of Vero cells cultured in FBS-supplemented and FastGro™ serum free media-supplemented over a six-day period. With an initial seeding density of 20,000 cells/cm², cultures maintained in Williams Medium E supplemented with 10% FastGro™ achieved a final cell density of 197,000 cells/cm², compared to 184,000 cells/cm² in the 10% FBS control.[68] This represents a 7% increase in both total cell yield and cell multiplication index when using chemically defined media like FastGro™.

These findings underscore the remarkable efficiency of serum-free formulations as viable, possibly superior, alternatives to serum-based systems.

Moreover, the widespread adoption of serum-free media in bioprocessing has rendered conventional trypsin redundant. Porcine derived, trypsin is a serine-protease enzyme that hydrolyses peptide bonds within proteins and between amino acids, exclusively at the C-terminal to Lysine and Arginine.[70] By this mechanism, Trypsin is used as a cell-dissociation agent- to dislodge cells prior to subculturing, cell counting, cryopreservation and any culture manipulation that requires the adherent cell to be "free" from its vessel. This step is called trypsinization.[85]

In the absence of FBS, which contains protease inhibitors like α1-antitrypsin capable of inactivating residual trypsin activity and buffering its cytotoxic effects, serum free media lacks such protective components. [85] Consequently, there is a growing reliance on animal-origin-free media and recombinant trypsin alternatives that provide gentler, more consistent detachment without compromising cell integrity or compatibility with chemically defined and animal-free systems.

TrypLE™, is one such alternative to conventional Trypsin. TrypLE™ is a recombinant fungal trypsin-like protease, which has proven effective at dissociating many different adherent mammalian cell lines.[45] In a comparative study, Tsuji et al. reported that while trypsin reduced the expression of several cell surface antigens, TrypLE™ preserved surface-antigen integrity, highlighting its gentle action during cell dissociation compared with the traditional enzyme.[91] Similarly, Accutase™ is a mild-acting, xeno-free dissociation reagent composed of proteolytic and collagenolytic enzymes.[46] Both products serve as direct replacements for conventional trypsin, with practical advantages such as eliminating the requirement for serum-mediated inactivation after passaging. By bypassing this step, TrypLE™ and Accutase™ enable gentler handling of cells and improve the overall efficiency of routine cell culture compared to traditional trypsin.[45, 46]

2.6. Analysing Future Prospects: Applications and Caveats of Serum Free Media & Xeno-Free Substitutes

The field of cell biology and cell engineering is undergoing a critical inflection point marked by the growing adoption of xeno-free systems in vaccine development. This paradigm shift is propelled by the superior safety profile conferred by eliminating non-human animal-derived components in both cell culture and downstream manufacturing processes.

Several factors are contributing to the adoption of xeno-free approaches in vaccine manufacturing. These include the broadening commercial availability of optimized media suitable for diverse cell lines, increased investment in scalable xeno-free platforms stimulated by global pandemic preparedness initiatives, and a growing regulatory preference for animal-component-free processes.[61] But despite these influences favoring serum-independent and chemically defined media, established serum-dependent cell culture methods are expected to retain their relevance and are unlikely to be entirely superseded in the near term. [96]

A primary impediment to the universal adoption of xeno-free systems is that not all cell lines currently utilized for vaccine production exhibit difficulty in adapting to serum-independent conditions. Certain lines, be it adherent or suspension, may display suboptimal proliferation kinetics or reduced viral yields when deprived of specific animal-derived components supplied by serum. This will henceforth necessitate extensive optimization of xeno-free media formulations before being able to achieve performance comparable to serum-dependent cultures.[35] Furthermore, the complexity

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and specificity of SFM and CDM formulations, while offering benefits in terms of consistency and safety, inherently limits its cross-usability across different cell lines. Consequently, the realization of a truly universal, "one-fits-all" xeno-free medium remains an elusive goal, at least for now.[44]

Nevertheless, significant advancements have been made to improve the performance of xeno-free media in bioproduction. In a study performed by Novo Nordisk Pharmtec, recombinant insulin was used to boost influenza virus production in HEK293SF-3F6 cells.[70] Said to stimulate anti-apoptotic and mitogenic pathways, insulin supplemented cultures showed increased viral titres of ~1.7-fold. [70] During infection, the influenza virus activates the PI3K/Akt pathway to facilitate viral entry and promote its replication and proliferation within host cells and in the presence of insulin, Akt phosphorylation was further increased, suggesting that insulin enhances influenza virus production by further activating the PI3K/Akt pathway. [70]

Beyond other benefits, implementing xeno-free alternatives in biopharmaceutical production of mAbs, vaccines, cell therapies, immunotherapies, and other human bioproducts prevents the risk of xenoimmunization—an immune response triggered in the recipient due to residual non-human, animal-derived components from the cell culture process present in the final therapeutic product. Minimizing this risk is impertinent for certain therapies where even minute amounts of foreign matter can elicit an unwanted immune outburst.[37]

While an immediate, wholesale shift to xeno-free systems in cell-culture vaccinology remains on the horizon, the trajectory is clear: expert consensus and compelling drivers point towards the inevitable elimination of animal-derived components across bioprocesses.

3. Conclusions: Embracing Change

In conclusion, this paper has sought to consolidate the major trends and nuances that define the modern landscape of cell culture-based vaccine production. As the saying goes, "change is the only constant" which proved to be true as the field of cell culture is going through a profound paradigm shift, not only in terms of cell culture media but also the vessels used to achieve high yield, cGMP-certified bioproducts.

Progressing from the early reliance on primary cell cultures, diploid cell strains and egg-based expression platforms, to adherent cell cultures, and then the increasing adoption of suspension systems for large-scale production- this initial shift has been particularly critical in responding to the urgent demands of global health crises. Furthermore, the advent of designer cell lines, in parallel with the integration of bioreactor systems, further optimized bioprocesses and has been instrumental in assuring vaccine production meets global demands.

Yet the most profound shift may be the deliberate move away from serum-dependent culture toward serum-free and xeno-free systems.[38] Animal derived components have a long-standing legacy and significance in culture media, but their legacy is now being dismantled in the pursuit of safer and more consistent alternatives.

The emergence of xeno-free practices represents the latest and arguably, most important advancement in cell biology and bioprocess engineering. The implementation of xeno-free methodologies goes beyond just risk reduction, encompassing ethical imperatives and aligning with stringent regulatory frameworks, thereby fostering the development of safer, more reliable vaccine manufacturing processes.

Looking ahead, the challenge is twofold: to harness multiomics-level insights to design media and cell lines that rival or surpass the productivity of serum-based systems, and to align these innovations with the industrial reality of pandemic preparedness, affordability, and equitable access. If achieved, the ability to cultivate cells robustly in fully defined, animal-free media will not just represent a technical refinement, it will mark a new era of vaccine and biologics production.

This transition from serum dependence to independence is not merely a technical advancement, it signifies a mature and increasingly sophisticated era in cell culture, poised to deliver the next generation of life-saving biologics.

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