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CELL-FREE BIOLOGICS PRODUCTION: THE FUTURE OF POINT-OF-CARE MEDICINE

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ABSTRACT

Biologic medicines have transformed the management of cancer, autoimmune disorders, infectious diseases, endocrine disorders, and rare diseases, but their manufacture commonly depends on centralized facilities, specialized cell culture infrastructure, extensive purification, controlled storage, and distribution networks. These requirements can constrain rapid deployment and access, particularly when demand is geographically dispersed or changes suddenly. Cell-free biologics production uses isolated transcription and translation machinery to synthesize proteins outside intact living cells, creating an open and programmable production environment. This review examines the scientific foundations, workflow, expression platforms, applications, enabling technologies, advantages, limitations, and regulatory considerations of cell-free biologics production, with particular emphasis on point-of-care and decentralized medicine. Cell-free systems can reduce the biological constraints associated with host-cell growth, enable rapid genetic reprogramming, support high-throughput experimentation, and facilitate freeze-dried formats that can be stored and activated when needed. Demonstrated applications include recombinant therapeutic proteins, cytokines, enzymes, antibodies and antibody-related products, glycoproteins, vaccines, antimicrobial proteins, and difficult-to-express proteins. Integration with synthetic biology, artificial intelligence, automation, microfluidics, portable reaction hardware, and

lyophilization may further support compact manufacturing platforms. However, cell-free production is not yet a universal replacement for conventional biomanufacturing. Important barriers include reaction cost, productivity and stability, folding, post-translational modification, purification, analytical control, sterility, reproducibility, scale-up, validation, and regulatory oversight. The future role of the technology is therefore likely to be complementary: centralized manufacturing will remain important for high-volume products, while validated cell-free platforms may expand rapid, distributed and personalized production for selected biologics and emergency settings.

KEYWORDS: Cell-free protein synthesis; Biologics; Point-of-care medicine; Synthetic biology; Personalized medicine; On-demand biomanufacturing.

INTRODUCTION

Biologic medicines are therapeutic products derived from biological sources or produced using biological systems and include recombinant proteins, monoclonal antibodies, vaccines, hormones, cytokines, enzymes, nucleic-acid-based products and other advanced biological modalities. Their clinical value is closely related to molecular specificity, the ability to replace or modulate biological functions, and the possibility of targeting disease mechanisms that are poorly addressed by conventional small-molecule medicines. At the same time, the structural complexity of biologics creates demanding manufacturing and quality requirements because sequence, higher-order structure, aggregation, purity and, for many products, post-translational modification can influence activity and safety.[1-2,13,46]

Conventional biologics production is predominantly centralized and often relies on engineered mammalian, microbial, yeast or other living production hosts. A typical workflow includes cell-line or strain development, expansion, bioreactor cultivation, expression, harvest, clarification, purification, formulation, filling and distribution. For complex therapeutic proteins, especially glycoproteins, process development can be extensive because production must simultaneously maintain host-cell health, product quality and process consistency. These systems are powerful and highly established, but the associated infrastructure, quality systems and logistics can create long lead times and fixed costs that are poorly matched to low-volume, rapidly changing or geographically dispersed demand.[3-5]

Centralized production also places substantial dependence on transportation and storage networks. Many biologics require controlled temperatures and carefully managed distribution because stability may be sensitive to heat, agitation, freezing or prolonged storage.

Interruptions related to transport, refrigeration, manufacturing capacity or shortages of critical materials can therefore affect availability. Although improvements in formulation, continuous processing and facility design can reduce some of these burdens, they do not eliminate the underlying dependence on large-scale production assets and complex supply chains.[2,6-7]

Cell-free systems address a different part of the manufacturing problem. Instead of placing the genetic program inside a living cell and waiting for cell growth, an operator supplies a DNA or RNA template directly to a reaction containing the molecular machinery required for transcription and translation. Because the reaction is open, individual components can be adjusted, removed or added without having to engineer and maintain a viable production organism. Modern cell-free protein synthesis systems can provide substantial protein yields and are increasingly used for rapid prototyping, difficult-to-express proteins, metabolic pathway testing, synthetic biology, and biomanufacturing research.[8-12]

The connection with point-of-care medicine is particularly important. A portable and on-demand manufacturing strategy does not imply that every therapeutic product should be manufactured beside the patient. Rather, it creates the possibility of moving selected stages of production closer to the site of need, using preprepared reagents or stabilized reaction systems that can be activated with a digital genetic template when a validated product is required. Demonstrations of freeze-dried cell-free systems have shown that biological synthesis can be moved into formats suitable for transport and use outside conventional laboratory infrastructure, supporting a vision of distributed biomanufacturing for selected applications.[13-14,16-17]

This review evaluates cell-free biologics production as an emerging pharmaceutical manufacturing paradigm rather than as a universal replacement for cell-based production. The objectives are to explain the scientific basis of cell-free protein synthesis, compare major expression platforms, review the production of important biologic classes, assess point-of-care opportunities, examine enabling technologies, and critically discuss technical, economic, quality, safety and regulatory barriers. Particular attention is given to the evidence required before decentralized production can be translated from experimental demonstrations into reliable clinical manufacturing.[15-16,36]

MATERIALS AND METHODS

This review was developed as a narrative scientific review focused on cell-free biologics production and its relevance to point-of-care medicine. Literature was identified primarily through PubMed-indexed records and established scientific journal sources, supplemented by

authoritative journal and regulatory webpages when necessary for current methodological or policy context. Search concepts included cell-free protein synthesis, cell-free gene expression, cell-free biomanufacturing, therapeutic proteins, glycoprotein synthesis, monoclonal antibodies, vaccines, lyophilized cell-free systems, portable biomanufacturing, point-of-care production, microfluidics, automation, synthetic biology, and quality or regulatory considerations. Priority was given to peer-reviewed research articles, major reviews, and recent literature, while landmark studies were retained when they established principles that remain relevant to the field.[8,11,13,16]

The evidence was organized thematically into: (i) biologic manufacturing context; (ii) principles and components of cell-free synthesis; (iii) production of representative biologic classes; (iv) point-of-care and decentralized use cases; (v) enabling technologies; (vi) benefits and limitations; (vii) quality and regulatory considerations; and (viii) future directions. Claims were framed conservatively, especially where the literature demonstrates technical feasibility but does not yet establish routine clinical manufacturing. The resulting synthesis is intended for academic review and educational discussion rather than as a substitute for product-specific development, validation or regulatory advice.[17-18,36]

RESULTS AND DISCUSSION

1. BIOLOGICS: CURRENT STATUS AND MANUFACTURING CHALLENGES

1.1 OVERVIEW OF BIOLOGIC MEDICINES

Biologics encompass a broad group of products in which the active substance is a biological molecule, a biological particle, or a product generated through biological processes. Recombinant proteins and monoclonal antibodies represent major therapeutic classes, while vaccines, hormones, enzymes, cytokines and newer nucleic-acid-based medicines expand the range of biological modalities. A defining feature of many biologics is the relationship between molecular structure and biological function: small changes in folding, aggregation, glycosylation or other modifications may change pharmacokinetics, potency or immunogenicity. [13,19-21]

Monoclonal antibodies are an important example because their clinical activity depends on a highly organized multidomain structure and, for many products, specific Fc-region glycosylation. Recombinant proteins such as growth factors, cytokines and hormones may be smaller than antibodies but can still present substantial folding or solubility challenges. Therapeutic enzymes may require correct active-site formation and cofactors, while vaccines can range from purified proteins and virus-like particles to more complex antigenic

constructs. Emerging products therefore differ greatly in the degree of cellular processing that must be recreated outside a living host.[1,22-24,29]

1.2 CONVENTIONAL BIOLOGICS MANUFACTURING

Traditional production commonly follows the sequence: cell line or strain development → cell expansion → bioreactor production → product expression → harvest → clarification → purification → formulation → packaging → distribution. Mammalian systems such as Chinese hamster ovary cells are particularly important for complex glycoproteins because they provide cellular machinery for folding, secretion and glycosylation. Escherichia coli and other microbial platforms can be faster and economical for many non-glycosylated proteins, whereas yeast, insect and plant systems occupy intermediate positions depending on the molecular requirements of the product.[1,3-4]

Large-scale conventional manufacturing offers mature process control and established regulatory pathways, but it requires infrastructure that is difficult to reproduce at every location. Bioreactors, controlled environments, seed-train development, environmental monitoring, chromatography and analytical testing all contribute to the manufacturing footprint. For high-demand products, these systems provide the capacity and consistency needed for commercial supply; their limitations become more evident when products are urgently required in small amounts, when patient-specific changes are needed, or when the target is difficult to express by a conventional host.[5,25-26]

1.3 LIMITATIONS OF CONVENTIONAL MANUFACTURING

A major limitation is the time and resource burden associated with establishing and maintaining a biological production process. Living cells must be expanded before they generate the desired product, and changes in productivity can arise from cell physiology, genetic stability, culture conditions and raw-material variability. Process-development activities may therefore be disproportionate to the scale of a short-term or geographically limited need. Conventional platforms also require substantial downstream processing because the product is generated within a complex biological mixture that contains host-cell proteins, nucleic acids, process-related impurities and, in some cases, aggregates.[3,5,27]

Infrastructure is another limitation. Large-scale bioreactors, cleanroom space, utility systems and purification equipment require capital investment and trained personnel. Distribution adds further requirements for controlled transport and storage, particularly for products that are temperature-sensitive. These characteristics do not make conventional manufacturing intrinsically inefficient; rather, they reflect an industrial model optimized for centralized,

predictable production rather than small-batch, rapidly reprogrammable production close to the point of care.[2,6-7]

The model can also be vulnerable to supply-chain disruption and production bottlenecks. A single manufacturing site may serve multiple markets, creating dependence on validated capacity, raw materials, specialized consumables and transportation. Pandemic response illustrates the tension between high-throughput centralized production and sudden changes in demand: rapid adaptation requires both biological development and manufacturing capacity. Decentralized platforms are attractive in this context because they could, in principle, distribute production capability instead of distributing only finished doses.[13,16-17]

2. FUNDAMENTALS OF CELL-FREE BIOLOGICS PRODUCTION

2.1 WHAT ARE CELL-FREE SYSTEMS?

A cell-free system reproduces one or more biological processes outside intact living cells. In cell-free protein synthesis, cellular membranes, growth requirements and most of the genome are removed while the molecular machinery required for gene expression is retained. The core process follows the same central biological logic as intracellular expression: a genetic template directs RNA synthesis and ribosomes translate that RNA into protein. The difference is that the reaction is performed in a controlled biochemical environment instead of inside a growing organism.[8,11,28]

The history of cell-free synthesis is linked to fundamental discoveries in molecular biology, including experiments that used cell extracts to understand how nucleic-acid information is converted into protein. Over subsequent decades, the field shifted from a primarily analytical tool to an engineering platform. Better extracts, energy systems, reaction design and genetic control increased protein output and broadened the range of molecules that can be synthesized. More recently, synthetic biology has enabled cell-free systems to function as programmable environments for rapid design-build-test cycles and distributed biomanufacturing.[9-10,28]

2.2 CELL-FREE PROTEIN SYNTHESIS

Cell-free protein synthesis contains the molecular components needed for transcription, translation and energy management. Depending on the platform, these components may be supplied through a crude lysate or as individually purified factors. A typical reaction contains a DNA or RNA template, ribosomes, transfer RNAs, aminoacyl-tRNA synthetases or related charging machinery, polymerases, translation factors, nucleotides, amino acids, salts, cofactors and an energy-regeneration system. The operator can alter concentrations or add

chaperones, redox reagents, membrane mimetics, glycosylation enzymes or non-canonical amino acids according to the target protein.[11,29-30]

Transcription converts DNA into messenger RNA using RNA polymerase, while translation uses ribosomes to decode the messenger RNA and assemble the amino-acid sequence. Because the system is open, these processes can be tuned independently and, in some formats, initiated directly from RNA without requiring a transcription step. This separation of biological processes makes cell-free systems useful for testing sequence variants rapidly, studying translation behavior, and producing proteins that would otherwise burden or kill host cells.[8,11,34]

Energy management is one of the central determinants of performance. ATP and GTP are consumed during translation, while transcription and amino-acid charging also require metabolic inputs. Modern reaction systems therefore use substrates such as phosphoenolpyruvate, creatine phosphate, maltodextrin or other regeneration strategies, often combined with engineered extract metabolism. Balancing energy supply with by-product accumulation is critical because excessive substrate loading does not necessarily improve output and may reduce reaction lifetime.[11,31,40]

2.3 TYPES OF CELL-FREE EXPRESSION SYSTEMS

Escherichia coli-derived systems are among the most widely used because they are relatively accessible, well characterized and capable of high expression rates. They are particularly useful for non-glycosylated proteins, enzymes, antibody fragments and proteins that are toxic to living bacteria. Wheat-germ and rabbit-reticulocyte systems provide eukaryotic translation machinery and can support selected proteins that are poorly produced in bacterial extracts. Insect and mammalian lysates can supply a more eukaryotic processing environment and may be useful when folding, secretion-associated processes or post-translational modifications become important.[11,29,32]

The PURE system, or protein synthesis using recombinant elements, replaces much of the complexity of a crude lysate with a defined set of purified components. This increases compositional control and makes individual variables easier to manipulate, but it can increase reagent cost and preparation burden. Plant-derived systems represent another promising class because they combine aspects of eukaryotic protein processing with scalable extract production; recent studies using tobacco BY-2 lysate have demonstrated native disulfide bonds and N-glycosylation alongside successful scaling of reaction volume.[33,38]

TABLE 1. COMPARISON OF DIFFERENT CELL-FREE EXPRESSION SYSTEMS.

Cell-Free System	Source	Advantages	Limitations	Suitable Biologics
E. coli extract	Bacterial lysate	High productivity; accessible; scalable	Limited eukaryotic PTMs; endotoxin considerations	Enzymes; cytokines; non-glycosylated proteins; fragments
Wheat germ	Plant germ extract	Good for some eukaryotic proteins; low protease background	Lower throughput; preparation cost	Research proteins; selected eukaryotic proteins
Rabbit reticulocyte	Eukaryotic lysate	Strong eukaryotic translation environment	Cost; limited scalability; product-specific constraints	Research proteins; selected eukaryotic proteins
Insect cell	Insect lysate	Eukaryotic machinery; useful for complex proteins	More complex preparation	Selected glycoproteins and membrane proteins
Mammalian lysate	Mammalian cells	Better support for disulfide bonds and some PTMs	Cost; complexity; scalability	Therapeutic proteins; selected glycoproteins and antibodies
PURE	Purified recombinant factors	Defined composition; precise control	High reagent cost; extensive preparation	Protein engineering; synthetic biology; controlled prototypes
Plant BY-2	Tobacco BY-2 lysate	Eukaryotic processing; scalable; native disulfide/glycosylation demonstrated	Reaction time and product-specific optimization	Antibodies; growth factors; VLPs; difficult proteins

3. COMPONENTS AND WORKFLOW OF CELL-FREE BIOLOGICS PRODUCTION

A practical cell-free workflow can be represented as: genetic template → cell-free reaction system → transcription and translation → protein folding and processing → purification → quality testing → final biologic product. The key engineering question is not simply whether a protein can be synthesized, but whether the resulting molecule can be produced reproducibly, purified to the required standard, and demonstrated to have the identity, purity, potency and stability expected of a therapeutic product.[15,36]

3.1 DNA AND RNA TEMPLATES

DNA templates may be plasmid-based, linear, PCR-derived or generated through amplification strategies, while RNA templates can be introduced directly to bypass transcription. Template design influences expression through promoter strength, untranslated regions, codon usage, mRNA stability and sequence architecture. Because a cell-free reaction does not require biological cloning between every design iteration, template variation can be tested rapidly, which is valuable for protein engineering and process optimization.[8,29]

3.2 CELL EXTRACT PREPARATION

Extract-based systems begin with a source organism or cell culture that is harvested, disrupted and processed to remove unwanted cellular structures and endogenous activities while preserving translation competence. Extract preparation is a significant source of variability because growth conditions, lysis procedures, clarification and storage can influence the final composition. Standardization of extract preparation is therefore necessary for reproducible production and is a central consideration for future manufacturing systems.[7,11]

3.3 ENERGY REGENERATION SYSTEMS

Energy regeneration systems maintain the nucleotide triphosphates required for transcription and translation and help extend reaction duration. Older systems often relied on relatively expensive high-energy substrates, whereas more recent approaches use metabolic pathways or low-cost feedstocks to improve economic performance. A manufacturing platform must evaluate not only peak yield but also reagent cost, reaction duration, waste generation and lot-to-lot reproducibility.[31,40]

3.4 TRANSCRIPTION AND TRANSLATION MACHINERY

The balance between transcription and translation determines how effectively a template is converted into product. Polymerase and ribosomal activity, messenger RNA stability, tRNA charging, initiation and elongation rates all affect productivity. Extract engineering can remove nuclease activity, reduce proteolysis, improve amino-acid recycling and redirect resources toward heterologous protein synthesis. Defined systems allow even finer control because individual translation factors can be changed independently.[11,29-30]

3.5 COFACTORS AND SUBSTRATES

Proteins may require metal ions, cofactors, reducing or oxidizing reagents, small-molecule ligands or other substrates to reach functional form. Cell-free synthesis is advantageous because these components can be supplied directly and their concentrations can be optimized without the transport and homeostatic constraints of living cells. The same flexibility,

however, creates a larger formulation design space that must be experimentally characterized for each product.[34]

3.6 PROTEIN FOLDING

Protein synthesis does not guarantee correct folding. Disulfide-containing proteins may need a controlled redox environment and disulfide isomerases, whereas hydrophobic membrane proteins may require detergents, liposomes, nanodiscs or microsomes. The ability to control these variables directly is a major advantage of cell-free systems, but it also makes the reaction more product-specific. Incorrect folding, aggregation or incomplete maturation can reduce functional yield even when total protein concentration is high.[29,35]

3.7 POST-TRANSLATIONAL MODIFICATIONS

Post-translational modifications are among the most important differences between simple recombinant proteins and complex therapeutic biologics. Glycosylation can influence stability, receptor binding, clearance and immune effector function. Conventional mammalian hosts perform these modifications through integrated cellular pathways. Cell-free platforms must either reproduce the relevant enzymatic machinery or use a hybrid strategy in which specific enzymes, membranes or organelles are added to the reaction. Research has shown that cell-free glycoprotein synthesis can be engineered using glycosylation pathways, but producing a clinically equivalent and consistently controlled glycan distribution remains challenging.[22,24,36-37]

3.8 PRODUCT RECOVERY AND PURIFICATION

After synthesis, the product must be separated from reaction components and process-related impurities. Cell-free reactions can simplify recovery because there is no intact biomass to remove, but the reaction still contains abundant proteins, nucleic acids, enzymes, metabolites and other components derived from the lysate or supplied reagents. Affinity tags, self-cleaving capture systems, chromatography, precipitation and membrane-based methods can be used depending on the product. For decentralized manufacturing, purification must be compatible with small footprints and robust operation, which may favor integrated capture strategies over multi-column industrial trains.[15,36,38]

3.9 QUALITY CONTROL

Quality control should establish identity, purity, potency, structural integrity, aggregation state and, where appropriate, glycosylation profile, residual host-derived materials, endotoxin, bioburden or sterility. A point-of-care platform cannot rely on the assumption that a validated upstream reaction guarantees a suitable final product. Instead, compact but

orthogonal analytical testing must be integrated with the manufacturing workflow so that each batch can be linked to predefined acceptance criteria before administration.[36]

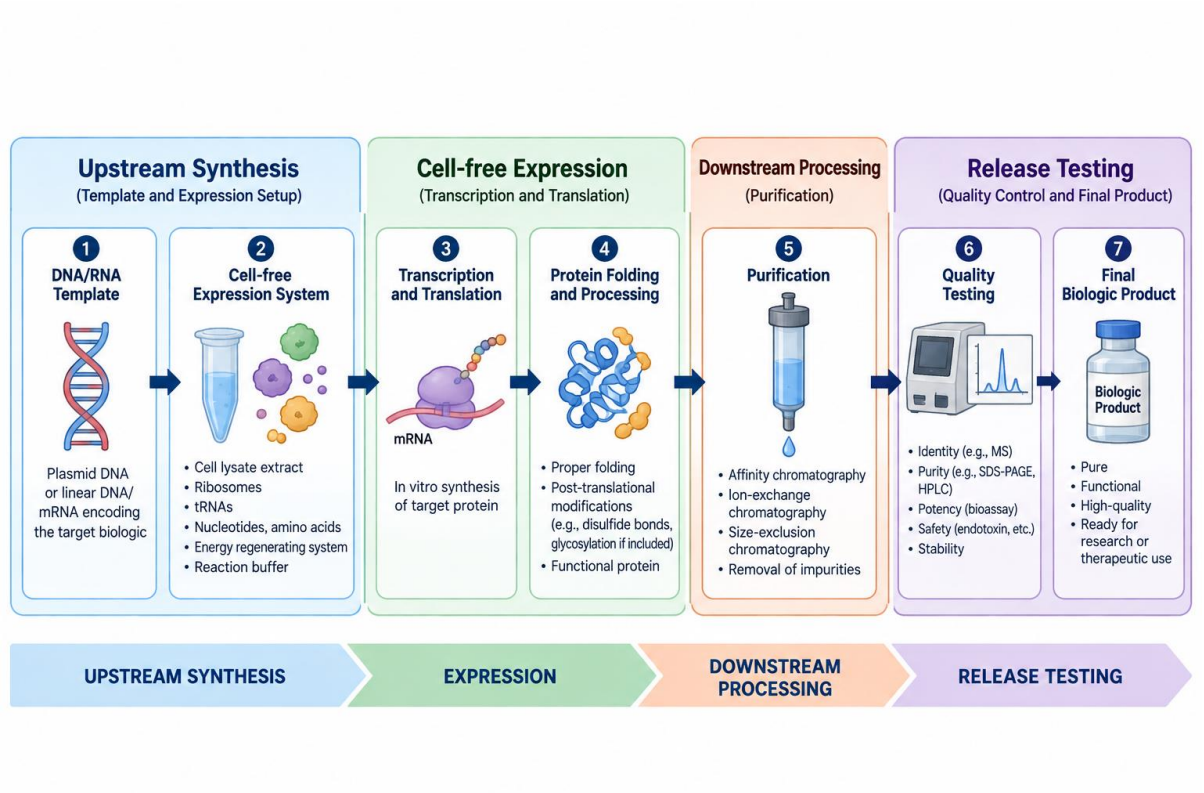


FIGURE 1. OVERVIEW OF CELL-FREE BIOLOGICS PRODUCTION WORKFLOW.

4. CELL-FREE PRODUCTION OF DIFFERENT BIOLOGICS

4.1 MONOCLONAL ANTIBODIES

Monoclonal antibodies are especially informative because they combine high clinical value with substantial manufacturing complexity. Their expression requires coordinated synthesis of heavy and light chains, assembly, disulfide-bond formation and, for many products, appropriate glycosylation. Cell-free systems can express antibody fragments and complete antibody-related structures and offer rapid template-to-protein cycles, which may be useful during discovery, screening and early process development.[29,36]

A more difficult goal is bedside production of an administration-ready monoclonal antibody. Demonstrations with mammalian lysate systems and other eukaryotic platforms indicate that cell-free synthesis can produce therapeutic proteins that require disulfide bonds and complex structures, while single-pot glycoprotein synthesis shows that glycosylation machinery can be incorporated into a reaction. Nevertheless, product heterogeneity, glycan control, downstream

purification and analytical release remain major barriers to routine point-of-care antibody manufacture.[15,36-37]

4.2 RECOMBINANT PROTEINS

Recombinant therapeutic proteins include cytokines, growth factors, hormones, replacement proteins and engineered scaffolds. Compared with antibodies, many of these proteins have simpler structural requirements and may therefore be attractive early targets for decentralized manufacturing. Open cell-free systems also permit rapid testing of sequence variants, fusion proteins and non-canonical amino-acid substitutions, supporting both protein engineering and production.[10,29,34]

A particularly relevant demonstration is cell-free production of granulocyte-macrophage colony-stimulating factor, where microscale optimization was connected to larger reaction volumes and downstream purification. Such work illustrates an important principle for point-of-care manufacturing: a useful platform must not merely produce protein in a laboratory tube but must maintain a predictable relationship between formulation, scale, yield and purification performance.[27]

4.3 THERAPEUTIC ENZYMES

Therapeutic enzymes may benefit from cell-free expression because the open system tolerates proteins that inhibit host-cell growth or are difficult to fold intracellularly. Cell-free approaches can also supply cofactors directly, which is useful for metalloenzymes and other catalytic proteins. Demonstrations involving streptokinase and serratiopeptidase show that cell-free systems can produce functionally relevant therapeutic proteins while avoiding some of the insolubility and refolding problems encountered in heterologous cellular expression.[15,24]

4.4 VACCINES

Cell-free synthesis can contribute to vaccine development at several stages. It can rapidly generate antigen variants for screening, support production of recombinant antigenic proteins, and provide a route to distributed manufacture of selected protein-based or particle-based vaccine components. Freeze-dried cell-free transcription-translation systems have been demonstrated as portable platforms capable of producing vaccine-related products and antimicrobial peptides after rehydration, supporting the concept of rapid response outside centralized facilities.[13,17]

The strongest value may be preparedness rather than routine replacement of established vaccine manufacturing. A digital template can, in principle, be changed more rapidly than a dedicated biological production strain, while stabilized reaction pellets reduce the need to

transport complete active manufacturing infrastructure. However, vaccines remain regulated biological products with strict potency, purity, safety and consistency requirements, so an emergency cell-free platform would still require validated assays and an appropriate regulatory framework before clinical use.[13,36]

4.5 CYTOKINES AND IMMUNE MODULATORS

Cytokines and immune-modulating proteins are attractive because many are secreted proteins for which rapid, small-batch production can be technically useful. Cell-free systems have demonstrated production of granulocyte-macrophage colony-stimulating factor and other disulfide-containing proteins, with reaction conditions that can be tuned to encourage productive folding. Their use at the point of care would still require dose accuracy and rigorous control of biological activity because cytokines can have narrow therapeutic windows and pleiotropic effects.[27,35]

4.6 ANTIMICROBIAL PROTEINS AND PEPTIDES

Antimicrobial peptides are useful model products for portable biomanufacturing because some are toxic to conventional production hosts. Removing the living-cell viability constraint can therefore expand the design space. Portable cell-free platforms have demonstrated production and functional validation of antimicrobial peptides using freeze-dried reaction pellets, showing how a stabilized manufacturing reagent can be transported and activated at the site of need.[13]

4.7 PERSONALIZED BIOLOGICS

Personalized biologics represent a longer-term opportunity. A future platform could use a patient-specific genetic or antigenic design and perform a small-batch synthesis within a validated manufacturing envelope. The advantage is not merely speed but reduced need to maintain many separate biological production strains. Nevertheless, personalization creates major quality and regulatory problems because every sequence change may create a new product profile, and therefore the manufacturing system would need to be validated as a controlled platform rather than validated only for one final molecule.[16,36-37]

5. CELL-FREE BIOLOGICS AND POINT-OF-CARE MEDICINE

5.1 DEFINITION OF POINT-OF-CARE MEDICINE

Point-of-care medicine refers broadly to healthcare delivery in which testing, decision-making or treatment occurs near the patient rather than exclusively within distant centralized facilities. In the context of biologics, the concept can be extended from point-of-care diagnostics to point-of-need production, where a therapeutic component is manufactured

close to the location at which it will be used. This should be distinguished from direct drug preparation by untrained personnel: a therapeutic manufacturing platform would require controlled inputs, qualified operators, validated procedures and release testing.[17,36]

5.2 DECENTRALIZED BIOMANUFACTURING

Decentralized biomanufacturing distributes selected production capabilities across multiple smaller facilities or devices. Cell-free systems are compatible with this model because the production machinery can be prepared centrally, stabilized and transported, while the final genetic template can be selected nearer to the time of use. This separates manufacture of the biochemical platform from manufacture of the product and could reduce some of the logistical complexity associated with moving finished, temperature-sensitive biologics.[13,17]

5.3 ON-DEMAND THERAPEUTIC PRODUCTION

On-demand production is particularly attractive when demand is intermittent or difficult to forecast. A stock of stabilized cell-free reagents can theoretically remain inactive until a validated template and formulation are introduced. The practical benefit depends on the shelf life of the components, the time required for synthesis and purification, and the ability to perform reliable quality checks before use. Therefore, “on-demand” should be understood as a manufacturing architecture, not a guarantee that every biologic can be safely made within minutes.[13,15]

5.4 PORTABLE BIO-MANUFACTURING PLATFORMS

Portable platforms seek to compress reaction, processing and analytical functions into a small footprint. Microfluidics can reduce reagent consumption and create controlled microenvironments, while automation can standardize liquid handling and timing. Freeze-dried reaction pellets or reagents can reduce dependence on continuous refrigeration and enable transport in a stable form. These features support a modular hardware architecture in which manufacturing can be scaled by running parallel small reactions rather than by building one large reactor.[13-14,32]

5.5 BEDSIDE PRODUCTION OF BIOLOGICS

Bedside production is conceptually most appropriate for selected products with relatively short manufacturing and purification workflows, sufficient stability, and assays that can be miniaturized. Experimental work with CHO lysates and mini-bioreactors has specifically discussed the feasibility of dose-level therapeutic protein production at the point of care. Such studies are important proof-of-concept steps, but translation into clinical practice requires

sterile design, validated raw materials, closed processing, contamination control and product release testing.[15,36]

5.6 REMOTE HEALTHCARE APPLICATIONS

Remote and rural healthcare settings may benefit when geographic distance makes centralized supply chains slow or unreliable. A small cell-free unit could, in principle, receive standardized reagent cartridges and digital manufacturing instructions, produce a selected protein locally, and perform a limited but sufficient quality-release workflow. The potential is strongest where transport infrastructure is weak and the required product is low-volume or intermittently needed. In practice, energy availability, water quality, operator skill and waste management would also have to be addressed.[13,17,27]

5.7 RURAL AND RESOURCE-LIMITED SETTINGS

Resource-limited settings highlight both the promise and the caution of the technology. A cell-free platform could eliminate the need for maintaining viable production cells and may reduce the footprint of some upstream activities, but it does not automatically eliminate the need for purified water, sterile consumables, analytical equipment or trained personnel. Cost reduction will therefore depend on system-level engineering rather than the cell-free reaction alone. The strongest models are likely to use standardized cartridges and shared infrastructure rather than fully independent manufacture of every material at every site.[13,36]

5.8 EMERGENCY AND DISASTER MEDICINE

Disasters and outbreaks create situations in which conventional distribution networks can become constrained while the need for selected medical products rises suddenly. Freeze-dried cell-free systems are attractive because the production machinery can be stored before an event and activated later. This architecture could support emergency production of selected antigens, antimicrobial peptides or protein reagents, provided that the target product, dosage form and analytical release system have already been validated for the platform.[13,16]

5.9 MILITARY AND FIELD MEDICINE

Field medicine presents a similar logistical problem but often with stricter constraints on size, weight, power and operator burden. Cell-free production can be inherently modular and can avoid the need to maintain live production cultures, making it a candidate for expeditionary biomanufacturing. However, ruggedization, contamination control, long-term reagent stability and simple release assays would be essential before practical deployment.[13,17]

5.10 SPACE MEDICINE

Space missions create an extreme form of distributed healthcare in which resupply is expensive or impossible and crew health needs may be uncertain. Cell-free manufacturing

offers an interesting architecture because stable reaction components can be transported in compact form and activated from stored information. The concept is still developmental, and radiation, thermal management, microgravity effects, payload constraints and crew time would need systematic evaluation. Its most realistic near-term role is as a research platform for demonstrating in-flight synthesis of selected proteins rather than as a complete pharmacy. [17,25]

TABLE 2. APPLICATIONS OF CELL-FREE BIOLOGICS PRODUCTION IN POINT-OF-CARE MEDICINE.

Healthcare Setting	Potential Application	Advantages	Challenges
Hospital	Small-batch therapeutic proteins	Local availability; reduced transport time	GMP/QC integration
Emergency unit	Urgent selected protein products	Rapid response	Sterility and release time
Rural clinic	Distributed production through cartridges	Reduced supply-chain dependence	Infrastructure and operator training
Pandemic response	Antigen/vaccine prototyping and selected production	Rapid reprogramming	Regulatory validation
Military/field	Portable protein manufacturing	Mobility and reduced logistics	Power, robustness, QA
Disaster/humanitarian	On-site production of selected biomolecules	Resilience during disrupted supply	Water, sterility, waste
Space medicine	Autonomous production of selected proteins	Reduced resupply dependence	Microgravity, shelf life, crew time

6. EMERGING TECHNOLOGIES ENABLING CELL-FREE BIOLOGICS PRODUCTION

6.1 SYNTHETIC BIOLOGY

Synthetic biology provides the design framework that makes cell-free platforms programmable. Genetic circuits, engineered promoters, optimized templates and pathway-level control can be tested without maintaining a permanent production organism. This shortens design-build-test cycles and enables reaction composition to be tuned for the target protein rather than inherited from a living host. Synthetic biology is therefore not an accessory to cell-free production; it is one of the main reasons the technology can function as a programmable manufacturing platform.[10,12]

6.2 ARTIFICIAL INTELLIGENCE AND MACHINE LEARNING

Artificial intelligence and machine learning could support cell-free manufacturing at several levels. Protein-design models can propose sequences with desired structural or functional properties, while predictive models can relate sequence features to expression, solubility or folding. Process models can also use experimental data to predict reaction performance and identify important variables such as magnesium concentration, temperature, template concentration or energy substrate levels. The value of AI is greatest when linked to closed-loop experimentation rather than used as a substitute for laboratory validation.[20-22]

For cell-free systems, AI may be especially useful because reactions can be run in small volumes and many components can be changed independently. Automated design-of-experiments can therefore generate dense datasets from comparatively small reagent quantities. The resulting models could support batch-specific adjustment of reaction conditions and early detection of abnormal performance. However, models trained on one extract source or instrument may not generalize to another, so robust datasets and defined quality attributes remain necessary.[20-22]

6.3 AUTOMATION AND ROBOTICS

Automation reduces operator-dependent variation and supports high-throughput screening. Liquid-handling robots can prepare reaction mixtures, initiate multiple genetic designs, collect time-course data and transfer products into downstream assays. In a future point-of-care device, automation could be used to constrain the manufacturing sequence so that only validated inputs are accepted and each batch follows a predetermined process. This could improve reproducibility, although hardware reliability and maintenance become new critical quality considerations.[22,32]

6.4 MICROFLUIDIC SYSTEMS

Microfluidics provides precise control of very small reaction volumes and can integrate mixing, reaction, separation and analysis on a single platform. Small volumes can reduce reagent consumption and shorten diffusion distances, while droplets or compartments can enable parallel experimentation. Reviews of CFPS formats increasingly describe microfluidics and related reaction architectures as routes toward integrated systems capable of performing several biological operations in sequence.[14,32]

6.5 LAB-ON-A-CHIP TECHNOLOGY

Lab-on-a-chip designs extend microfluidics by integrating reaction and analytical functions into compact devices. For biologics manufacturing, an ideal device might combine template introduction, expression, purification and quality assessment. The main challenge is that each

function must work reliably at the scale of the intended therapeutic dose, not merely demonstrate analytical performance. Materials compatibility, adsorption, clogging, heat management and reagent storage are therefore important engineering issues.[14,32]

6.6 PORTABLE BIOREACTORS

Cell-free reactions do not require conventional growth bioreactors, but they may still benefit from controlled mixing, oxygen transfer, temperature regulation and feed strategies. Small reactors and rocking systems can support better control than static tubes and may provide a route between laboratory-scale reactions and integrated devices. Recent scaling studies with eukaryotic lysates demonstrate that cell-free reactions can be extended over several orders of magnitude while retaining useful productivity, although clinical manufacturing scale is a separate regulatory and engineering question.[33]

6.7 FREEZE-DRIED CELL-FREE SYSTEMS

Lyophilization is one of the most important technologies for field deployment. Freeze-dried cell-free reactions can be prepared centrally, stored and rehydrated when needed. This format removes the requirement to maintain active biological machinery during transport and supports a manufacturing model in which the reaction is effectively a shelf-stable kit. The practical value depends on preservation of activity, protection from humidity and temperature excursions, package integrity, and the ability to reproduce performance after long storage.[13,17,23]

6.8 DIGITAL AND PROGRAMMABLE BIOLOGY

Programmable biology connects digital instructions with physical biological production. Rather than shipping a different living production strain for every product, a platform could receive a standardized sequence file and use it to configure a validated reaction. This could simplify inventory and support rapid adaptation, but it also introduces cybersecurity, data integrity and sequence-identity considerations. Any clinically relevant platform would need controls ensuring that only authorized and validated manufacturing instructions can generate released product.[36-37]

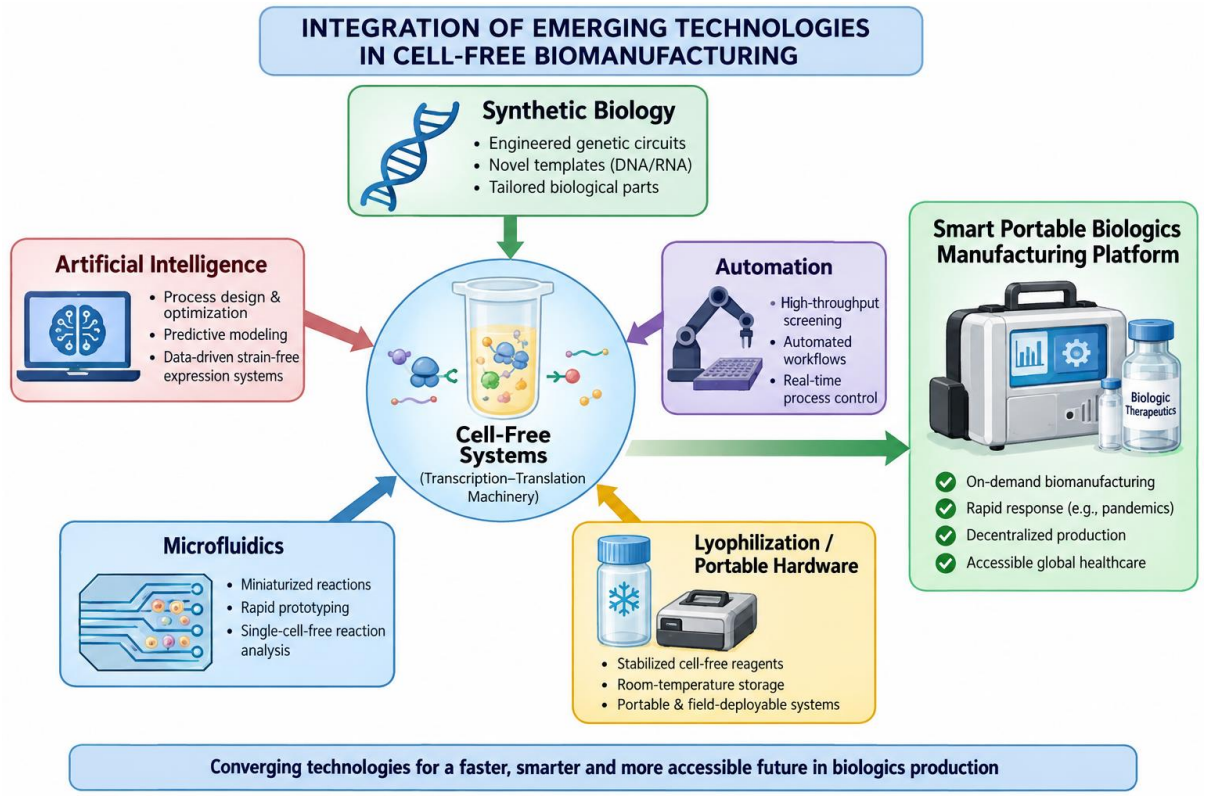


FIGURE 2. INTEGRATION OF EMERGING TECHNOLOGIES IN CELL-FREE BIOMANUFACTURING.

TABLE 3. EMERGING TECHNOLOGIES SUPPORTING CELL-FREE BIOMANUFACTURING

Technology	Role	Potential Impact
Synthetic biology	Sequence and circuit design	Fast reprogramming
AI/ML	Prediction and optimization	Improved yield and fewer experiments
Robotics	Automated reaction setup	Reproducibility
Microfluidics	Small-volume reaction control	Lower reagent consumption
Lab-on-chip	Integrated production and analysis	Portable manufacturing
Lyophilization	Long-term reagent stabilization	Field deployment
Digital manufacturing	Template-driven recipes	Distributed platform control

7. ADVANTAGES OF CELL-FREE BIOLOGICS PRODUCTION

The central advantage of cell-free synthesis is speed and flexibility. A reaction can be programmed directly with DNA or RNA without the cloning, transformation and cell-growth steps required for many cell-based workflows. This makes cell-free systems well suited to rapid expression screening and to proteins that impose a metabolic burden or toxicity on host

cells. The open reaction environment also allows direct manipulation of folding, cofactors and reaction chemistry.[8,10-11]

A second advantage is the possibility of decentralized production. Freeze-dried systems can be transported as stabilized manufacturing reagents, and small-volume reactions can be run in parallel. This architecture may reduce reliance on centralized factories for selected low-volume or emergency products. It may also reduce some aspects of cold-chain dependence when the manufacturing reagents themselves can be stored under less demanding conditions, although the final biologic may still require controlled storage.[13,16-17,23]

Cell-free synthesis also provides a powerful platform for personalization and rapid response. New genetic variants can be tested quickly, rare proteins can be produced without establishing dedicated cell lines, and reaction parameters can be computationally optimized. However, these advantages should be interpreted as platform capabilities rather than evidence that any individual cell-free process is automatically cheaper or safer than its conventional equivalent. The commercial and clinical value depends on the total system, including reagents, purification, analytics, hardware and regulatory requirements.[15,20,36]

Table 1 highlights that the principal distinction is architectural rather than simply quantitative. Cell-free production is potentially more modular and rapidly reprogrammable, whereas conventional systems remain stronger in mature large-volume manufacturing, established scale-up and validated regulatory pathways. The two approaches are therefore more likely to coexist than to compete across every biologic product category.[15-16,36]

8. CHALLENGES AND LIMITATIONS

8.1 LOW PROTEIN YIELD

Although modern cell-free systems can achieve high titers for selected proteins, performance remains highly target dependent. Difficult proteins may consume substantial reaction resources without reaching a functional form. Yield can also decline as reaction components become depleted or inhibitory metabolites accumulate. A platform intended for therapeutics therefore needs product-specific productivity benchmarks rather than relying on a single headline maximum.[8,11,34]

8.2 HIGH PRODUCTION COSTS

Cost remains a major barrier because purified enzymes, nucleotides, amino acids, cofactors and energy substrates can be expensive. Extract-based systems can reduce some costs but introduce batch variability and additional processing. Cost models must include the entire process, including template preparation, cartridges, purification, analytics, waste disposal and

operator time. For high-volume biologics, conventional fermentation or cell culture may remain economically superior.[5,15,36]

8.3 LIMITED REACTION STABILITY

Cell-free reactions are finite biochemical systems. Activity decreases as essential substrates are depleted, inhibitors accumulate and macromolecules lose function. Continuous-exchange and fed-batch formats can extend reaction duration, but additional hardware increases complexity. For portable devices, long reaction stability must be balanced against simplicity, cost and storage requirements.[11,31]

8.4 PROTEIN FOLDING CHALLENGES

Correct folding is a recurring limitation, particularly for multidomain proteins, disulfide-rich proteins and membrane proteins. The cell-free environment permits the addition of chaperones, redox systems, lipids or microsomes, but these interventions can complicate formulation. Total protein yield therefore cannot be used as a surrogate for therapeutic product yield.[29,35]

8.5 POST-TRANSLATIONAL MODIFICATIONS

Many therapeutic proteins need post-translational modifications that are difficult to recreate in a minimal reaction. Phosphorylation, acetylation, lipidation and glycosylation may require additional enzymes or organellar systems. For a drug product, the challenge is not merely to produce a modification but to reproduce the required degree and distribution of that modification consistently.[22,24,36-37]

8.6 GLYCOSYLATION CHALLENGES

Glycosylation is particularly important because glycan structures can affect activity, stability, serum half-life and immunological properties. Cell-free glycoprotein synthesis has advanced through glycoengineering and incorporation of glycosylation machinery, but the systems remain more complex than non-glycosylated protein synthesis. For many antibody products, conventional mammalian manufacturing therefore retains a major advantage in established and characterizable glycan processing.[22,24,36-37]

8.7 SCALE-UP DIFFICULTIES

Cell-free reactions can scale surprisingly well, but scale-up is not always a simple multiplication of volume. Oxygen transfer, mixing, heat management, reaction geometry and component gradients can change with scale. Thin-film systems and specialized mixing strategies have been developed to improve productivity, while eukaryotic lysate studies have demonstrated linear scale-up across substantial volume ranges. Nevertheless, manufacturing-scale validation requires more than preserving volumetric yield.[1,26,33]

8.8 PRODUCT PURIFICATION

Downstream processing may become the dominant step when the target concentration is low or when the reaction contains many impurities. Cell-free synthesis eliminates biomass, but not molecular complexity. A decentralized platform may need integrated affinity capture, membrane separation or a simplified purification cartridge to remain practical. Each additional operation creates opportunities for product loss, contamination and variability.[15,38]

8.9 PRODUCT STABILITY

Even when the manufacturing process is successful, the final biologic must remain stable through storage and administration. Cell-free synthesis does not automatically solve formulation problems, and some therapeutic proteins may still require refrigeration. A realistic decentralized model therefore combines stable manufacturing reagents with product-specific formulation science rather than assuming that all cold-chain requirements disappear.[6,13]

8.10 QUALITY CONTROL

Quality control becomes more demanding when manufacturing is distributed. Centralized facilities can concentrate analytical expertise and equipment, whereas point-of-care systems may have limited laboratory resources. Compact assays must still demonstrate identity, purity and potency with adequate sensitivity and specificity. This favors platform designs in which analytical testing is built into the device rather than added as an external activity.[36]

8.11 REGULATORY CHALLENGES

Regulatory systems are generally organized around defined products and controlled manufacturing processes. A decentralized platform that changes its genetic template while retaining a common production architecture challenges conventional assumptions about site, process and product comparability. Regulatory acceptance may therefore require a platform-based framework that defines validated operating ranges, allowable inputs, critical quality attributes and change-control rules.[36-37]

8.12 SAFETY CONCERNS

Safety concerns include microbial contamination, endotoxin, residual nucleic acids or host-cell proteins, process-component impurities, incorrect folding, aggregation and unintended biological activity. The absence of intact cells does not make a process automatically sterile or clinically safe. Biosafety also includes handling of genetic templates and prevention of unauthorized or erroneous manufacturing instructions.[36-37]

TABLE 4. MAJOR CHALLENGES AND POSSIBLE SOLUTIONS.

Challenge	Impact	Possible Solution
Protein folding	Inactive product	Redox tuning; chaperones; microsomes
Glycosylation	Heterogeneous critical quality attributes	Engineered glycosylation pathways; glycoanalytics
Reaction cost	Poor economics	Low-cost energy regeneration; process intensification
Batch variability	Reduced reproducibility	Standardized extracts or defined systems
Product purification	Complex workflow	Integrated affinity/membrane purification
Analytical release	Point-of-care delay	Miniaturized orthogonal assays
Regulatory uncertainty	Slow adoption	Platform-based validation and change control

9. REGULATORY AND QUALITY CONSIDERATIONS

Therapeutic cell-free manufacturing must meet the same fundamental expectations applied to other biologics: the product should have defined identity, purity, potency, quality and safety characteristics, and the process should operate under appropriate quality systems. Good Manufacturing Practice principles remain relevant even when production occurs in a compact or decentralized format. A smaller reactor does not imply a smaller quality requirement.[36]

Critical quality attributes should be defined for each product and may include primary sequence integrity, higher-order structure, aggregation, charge heterogeneity, glycosylation, residual host-derived material, endotoxin, sterility or bioburden, and biological potency. Process parameters should be linked to these attributes so that changes in template, extract lot, temperature or reaction composition can be evaluated using a risk-based control strategy. This is especially important in platform manufacturing because many final products may share common hardware but differ in molecular requirements.[36-37]

Sterility and contamination control require particular attention in point-of-care settings. Closed reaction cartridges, sterile single-use fluid paths and controlled reagent packaging could reduce operator exposure and contamination risk. However, a decentralized process would still need validated sterilization or aseptic assembly strategies and a release test appropriate to the product. A portable device that can synthesize protein but cannot reliably establish product safety would not be suitable for clinical administration.[36]

Regulatory pathways will also need to address distributed manufacturing, software-controlled recipes and platform changes. For example, a system may use the same validated reaction architecture to produce different proteins by changing a genetic template. Regulators would need to determine which elements define the platform, which elements define the product,

and how comparability is demonstrated after changes. FDA and EMA expectations for biologics emphasize control of product quality and manufacturing processes; decentralized cell-free systems therefore need to fit within, rather than bypass, established principles of pharmaceutical quality.[36,38]

10. APPLICATIONS IN DIFFERENT HEALTHCARE SETTINGS

10.1 HOSPITALS

Hospitals are a logical intermediate setting between centralized factories and true bedside manufacturing. A hospital-based cell-free platform could support limited production of selected proteins or reagents when centralized supply is delayed or when patient-specific demand is small. Integration with hospital pharmacy, microbiology and quality systems would be necessary, and the preferred operating model may be a centralized hospital biomanufacturing unit serving several clinical departments rather than individual bedside devices.[17,36]

10.2 INTENSIVE CARE UNITS

In intensive care, therapeutic demand can change rapidly and patient conditions can evolve faster than centralized manufacturing schedules. The strongest near-term application may be rapid preparation of research or diagnostic proteins rather than routine manufacture of fully regulated therapeutic doses. Any therapeutic use would require validated dose control, sterility and release testing, making the ICU more suitable as a future deployment environment than as an immediate target.[36]

10.3 RURAL HEALTHCARE

Rural healthcare may benefit from reduced dependence on long supply chains for selected low-volume biologics. Portable systems could be deployed through regional hubs, with standardized reagents and remote technical support. Practical implementation would depend on electricity, environmental control, trained staff and reliable quality testing, so networked regional manufacturing is likely to be more realistic than fully autonomous rural production.[13,17]

10.4 EMERGENCY MEDICNE

Emergency settings may require rapidly available protein reagents or selected therapeutics. Portable cell-free systems are attractive because reaction components can be stored in advance and activated when required. The technology is therefore particularly relevant to preparedness planning, but product selection should be narrow and evidence-based rather than assuming that any biologic can be made safely during an emergency.[13,16]

10.5 PANDEMIC RESPONSE

Pandemics create uncertainty in pathogen identity, antigenic drift and demand volume. Cell-free systems can accelerate antigen prototyping and, potentially, support distributed production once a validated target is known. Their greatest value may therefore be in bridging the gap between genetic sequence availability and manufacturable protein, complementing rather than replacing high-throughput centralized vaccine or therapeutic production.[13,16]

10.6 PERSONALIZED MEDICINE

Personalized medicine could use cell-free platforms to produce patient-specific proteins, antigens or protein-based reagents after a validated design process. The major challenge is that personalized manufacture increases product variability by design. Strong digital traceability, sequence verification and platform-level validation would be essential.[20,37]

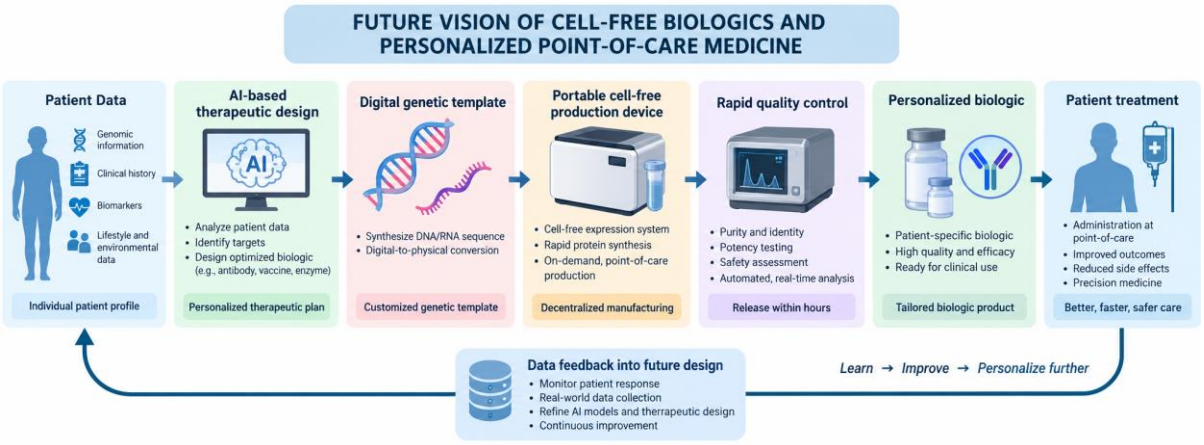


FIGURE 3. FUTURE VISION OF CELL-FREE BIOLOGICS AND PERSONALIZED POINT-OF-CARE MEDICINE.

10.7 RARE DISEASE TREATMENT

Rare diseases can present small patient populations and limited commercial incentives for large inventory manufacture. A flexible cell-free platform could theoretically reduce the need for dedicated large-scale facilities for some replacement proteins. However, clinical economics and regulatory requirements would still determine whether decentralized manufacture is more practical than specialized centralized production.[15,36]

10.8 MILITARY MEDICINE

Military medicine values mobility, resilience and autonomy. Cell-free platforms align with these objectives by allowing biological production without maintaining live cultures. Their

deployment would require rugged hardware, low power demand, long reagent shelf life and robust quality release under field conditions.[13,17]

CONCLUSION

Cell-free biologics production represents a promising shift from biologics manufacturing based exclusively on living production organisms toward a more programmable and modular biochemical approach. By separating protein synthesis from cell growth, cell-free systems offer rapid genetic reprogramming, flexible reaction control and opportunities for decentralized or on-demand production. These capabilities are particularly relevant to point-of-care medicine, emergency response, remote healthcare and potentially personalized therapeutic manufacturing. The field has already demonstrated production of diverse proteins and other biologically active products and has benefited from advances in synthetic biology, eukaryotic extracts, glycoengineering, microfluidics, automation and lyophilization.

Despite these transformative capabilities, cell-free biomanufacturing faces significant scientific, economic, and regulatory hurdles that prevent it from serving as an immediate, wholesale replacement for established industrial fermentation. Chief among these challenges are high raw material and reagent costs, limitations in long-term reaction stability, and the complexities of achieving correct macromolecular folding, authentic post-translational modifications, and native glycosylation profiles at scale. Downstream hurdles—including continuous purification, rigorous analytical batch testing, large-scale volumetric scaling, maintaining sterility in open formats, and ensuring batch-to-batch reproducibility—further compound these operational difficulties. Compounding the technical hurdles is a regulatory landscape primarily structured around centralized, fixed-facility manufacturing, which currently lacks the standardized frameworks needed to validate programmable, distributed, or point-of-use production units. At the same time, cell-free synthesis should not be portrayed as a universal replacement for conventional biologics manufacturing. Cost, reaction stability, folding, post-translational modification, glycosylation, purification, analytical testing, scale-up, reproducibility, sterility and regulatory control remain substantial barriers. The most credible path forward is a complementary model in which centralized manufacturing continues to serve mature high-volume products while validated cell-free platforms address selected low-volume, urgent, distributed or personalized needs. Future progress will depend on reducing reagent costs, improving product quality, integrating robust quality control and building regulatory frameworks that can accommodate programmable and decentralized manufacture. With these developments, cell-free biologics production could become an

important component of a more resilient, responsive and patient-centered pharmaceutical manufacturing ecosystem.

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