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Current Opinion in Chemical Engineering

Cell free protein synthesis: a viable option for stratified medicines manufacturing?

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Stratified medicines are defined as medicines which target diseases where the patients have been preselected for treatment based on their response to a diagnostic test. The pipeline of these medicines cover a wide range of different treatment types including cell and gene therapies, vaccines based on peptides or proteins; and protein based therapies. These increasingly diverse and by definition smaller market size products require improved agility and productivity in process design if manufacture and supply of affordable medicines is to be achieved. In this paper we review the current state of cell free synthesis (CFS), the new technologies and strategies being developed and its application to the production of stratified medicines; focusing on the production of protein based therapeutic products.

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Current Opinion in Chemical Engineering 2017, **18**:77–83

This review comes from a themed issue on **Biotechnology and bioprocess engineering**

Edited by **Nigel J Titchener-Hooker**

For a complete overview see the Issue and the Editorial

Available online 15th November 2017

https://doi.org/10.1016/j.coche.2017.10.003

A brief history of cell free synthesis systems

Cell-free protein synthesis systems were first developed more than 50 years ago as a tool to investigate the genetic code [1] and have been widely applied for synthesis of proteins for structural biology (e.g. [2,3]). Until recently, the main commercial systems available included wheat germ extract, Sf21 insect cell extracts, rabbit reticulocyte lysate, and *E. coli* extracts based on the T7 or SP6 promoter system [4]. However, there has been a proliferation of research activity in the past decade, leading to improvements in yield from existing systems [5–9], as well as the development of extracts based on diverse hosts such as *Saccharomyces cerevisiae* [10,11], various *Streptomyces* species [12,13], *Brevibacillusfdx brevis* [14], *Bacillus subtilis* [15], *Gluconacetobacter hansenii* [16], *BY-2* Tobacco cells [17], Chinese Hamster Ovary cells [18••], HeLa cells [19] and the K562 human leukaemia cell line [5]. Extracts can be engineered to promote protein folding and disulphide bond formation [20] and N-linked glycosylation can be achieved by including the microsomal fraction [5,18••] or oligosaccharide transferases [21] in the extracts. Systems have also been engineered for efficient incorporation of non-natural amino acids in order to provide a handle for additional chemical modification. In one such example, the site-specific incorporation of a non-natural amino acid was used to synthesise an antibody–drug conjugate with unprecedented homogeneity [22••]. The synthesis of a range of industrially relevant proteins has been demonstrated including antibodies and antibody fragments [20,23••,24–26], vaccine candidates [27,28] and other therapeutic proteins [29,30].

Cell free synthesis as a new manufacturing platform?

Historically biological medicines have been produced by live cell fermentations where, as the name suggests, live cells are used to produce the product. In this model the reagent (cell) generation step is intricately linked to the product generation, and is usually run from a centralised facility because of the complex infrastructure needed to amplify and maintain live cells. Because of the temperature sensitivity of most biological recombinant medicines and demanding regulatory framework, a sophisticated cold chain and distribution system is often required to get the medicines to the treatment sites, which can deny patient access to communities which lack these established distribution networks. The complex production model also often results in a manufacturing time line which is much longer than the patient treatment timeline, adding additional costs and often restricting patient access.

A typical CFS is made up of a crude cell extract which is usually obtained by lysing the cell and using centrifugation to remove structures and components not necessary for protein production. An energy source and the DNA of the protein to be expressed are then added. Depending on the source and nature of the cell extract other cofactors and supplements such as ribosomes, tRNA synthetases, translation initiation and elongation factors, among others, may be needed. Because the process uses biological components but without the use of living cells, the product synthesis step can be separated from the reagent (crude lysate) generation, increasing process agility, and

resulting in a process that is more akin to a chemical reaction.

This 'simplified' product synthesis step requires little of the complex infrastructure normally associated with a centralised live cell facility model, and lends itself to a distributed manufacturing model where the drugs are made at the point of treatment. A move to a distributed manufacturing model using CFS, and with the emergence of lyophilised cell extracts [31] which could negate the need for complex cold chain distribution networks entirely, has the potential to reduce the extended manufacturing time line we have grown accustomed to from live cell processes to one similar to the patient timeline, whilst also improving patient access, particularly to communities without established distribution networks. More importantly in the context of stratified medicines, such a move would enable the on demand synthesis of small batches of product.

However a switch to a distributed manufacturing model brings the issue of quality control to the fore if regulatory expectations surrounding complex release assays [35] for every batch of drug substance remain in place. This is a significant issue as the infrastructure for testing at multiple sites would negate the other benefits of a distributed manufacturing model. It has led some to propose entirely different models of drug supply to produce such medicines in an affordable manner [35]. A possible solution to the issue of testing at multiple sites is the role of automation in CFS. The relatively simple, well defined processes of CFS lend themselves to automation and the development of well validated models. It is possible to imagine a future state where the use of newer online monitoring techniques combined with the repeatability resulting from automation could reduce the need for onsite testing. As long as the process parameters/setpoints remains within a predefined parameter space where data exists to show that CQA are met, a case could be made for a reduction, or even elimination, of the onsite testing regime. The need for innovation in the analytics space is not a new idea and has been recognised before, most notably in the FDA's Process Analytical Technology initiative. Figure 1 is a graphical summary of the differences between a typical current manufacture and supply model to a CFS model which incorporates distributed manufacturing, leveraging the agility and flexibility inherent to CFS.

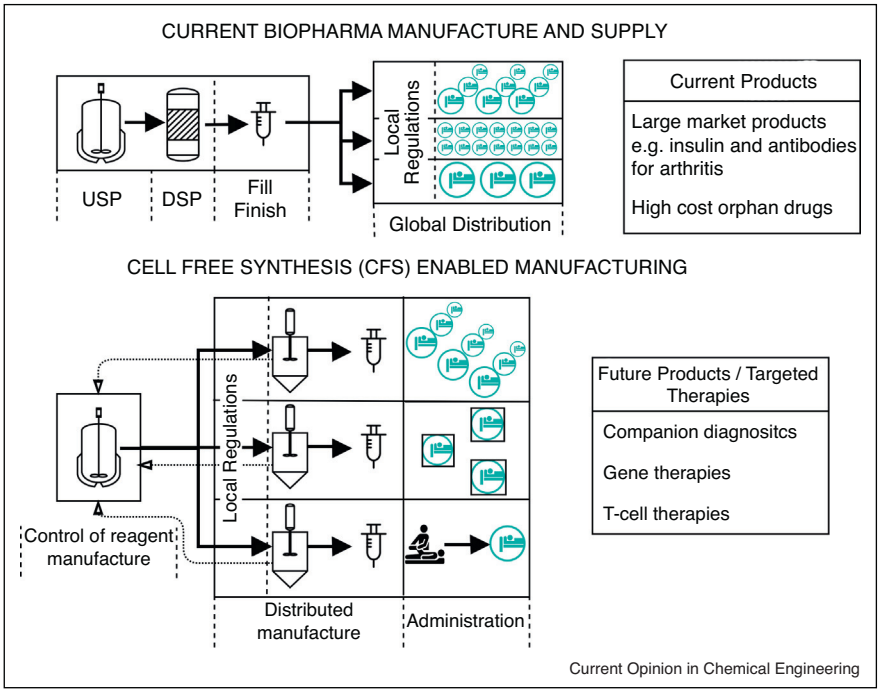
Survey of stratified medicine production ready CFS systems

As stated earlier there are a number of different types of CFS systems currently available including *E. coli*, insect cells, rabbit reticulocytes, CHO, yeast, and a few other more obscure ones. Although each system has been shown to be capable of producing active recombinant products we have performed a SWOT analysis on the systems we think are most suitable for stratified medicines production (Table 1). Each system currently has its advantages and disadvantages in terms of cost effectiveness, potential maximum yield, and the ability to make more complex (with respect to post translational modification (PTM) or multi-unit) products, but in general as the complexity of the product increases so does the complexity/cost of production, to the point where CFS is no longer competitive when compared to the comparable live cell fermentation systems. Process complexity can be linked to the nature of the product and the type of CFS selected is made on a case-by-case basis. For example products that require significant PTM like glycosylation (e.g. mAbs) may require the use of CHO based CFS, while simple vaccines may be made in *E. coli*-based CFS systems which have minimal post translational capacity, but can be modified to enhance protein folding and disulphide bond formation. Yeast based systems are somewhere in between. Indeed, considerable progress has been made in the cell engineering of yeast to create functional glycosylation pathways [32].

In all cases the challenge to CFS is most likely to come from the equivalent live cell process. The area in which CFS has a distinct competitive advantage is in process agility and potential for distributed manufacture in hospitals or even at the bedside. Processes based on live cell's involve long lead times for the development and validation of a stable cell lines before the start of manufacturing. Additionally, once in production the quality and efficacy of the drug substance, described by its Critical Quality Attributes (CQA) can be very sensitive to process conditions. This necessitates holistic control strategies and extensive analytical support to achieve robust manufacture of these products where live cells present significant risks of unacceptable batch-to-batch variation. The current dominant industry model of a centralised production facility where the processes can be tightly controlled is a direct consequence of this need for significant infrastructure investment and a highly skilled workforce. Although this system has its advantages it does require the generation and maintenance of a complex seed generation chain, the holding of large inventories of raw and in process materials, and a sophisticated cold chain requiring product to have a shelf life in excess of 12 months. These factors result in a lack of agility and manufacture typically being centralised in one or two sites globally.

If as is widely believed increased understanding of disease will give rise to personalised approaches to medicine, driving the need for stratified medicines, that is, a greater array of therapeutics directed and smaller patient populations to improve clinical outcomes, it will place great stress on this model of manufacture and supply. Perhaps even more importantly, process development resources for this increased portfolio of products both in term of

Figure 1



Comparison of a current biopharma manufacture and supply process to a cell free synthesis enabled platform based on a distributed manufacturing model.

Table 1

SWOT (Strength–Weaknesses–Opportunity–Threats) analysis of different types of cell free protein synthesis systems.

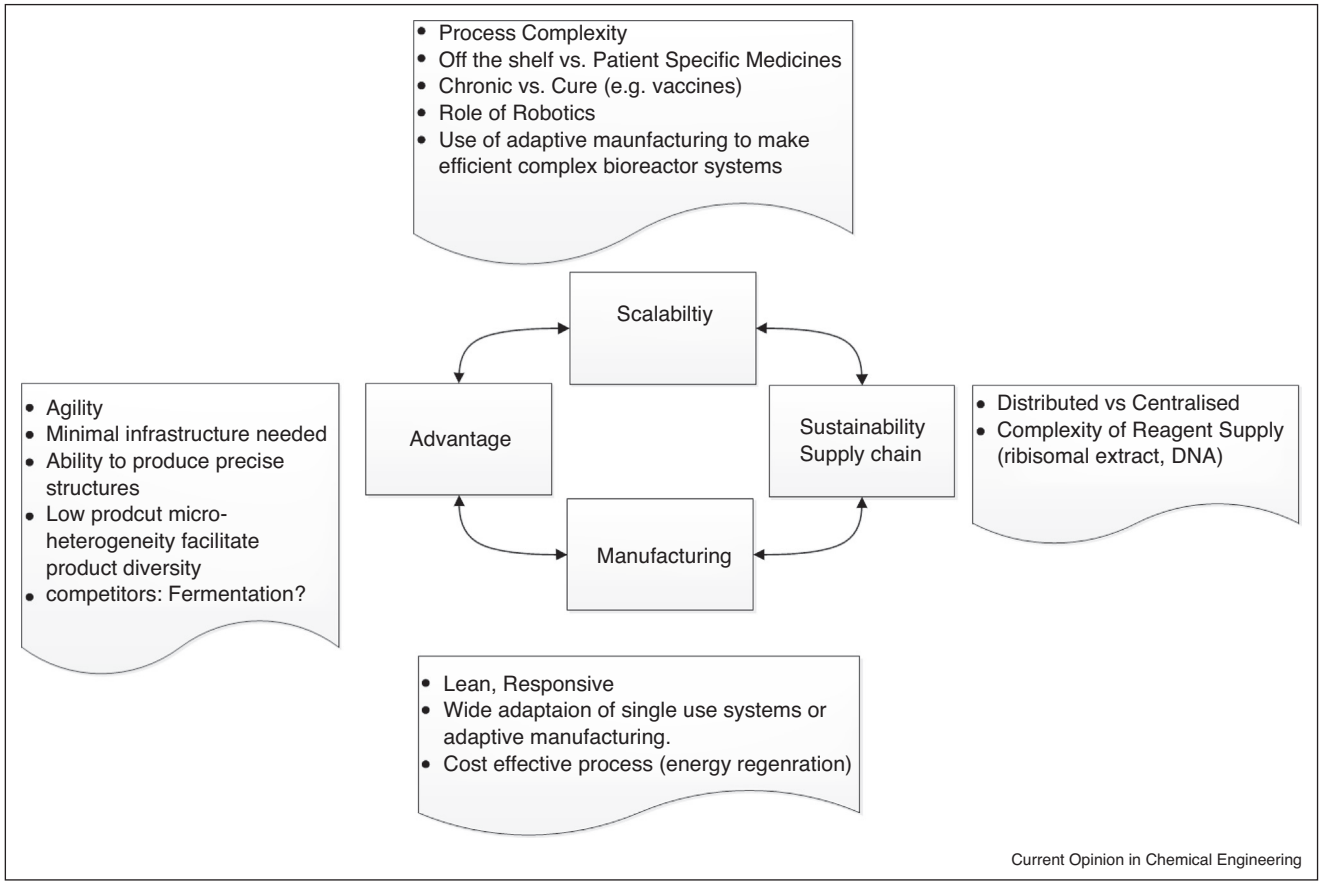
E. coli	Yeast	CHO
☐ *Strengths* — established cell based platform for biopharma, established routes for non-natural amino acid incorporation for ADC and other applications, agility	☐ *Strengths* — wide range of possible PTMs, low/lack of endotoxins	☐ *Strengths* — confidence and knowledge of use in mAb manufacture
☐ *Weaknesses* — few PTMs, no glycosylation, availability/IP	☐ *Weaknesses* — under developed, control of PTMs, less used as a host cell for manufacturing	☐ *Weaknesses* — cost, need for supply of intermediates during synthesis
☐ *Opportunities* — synthetic biology strategies to engineer PTMs.	☐ *Opportunities* — development of controlled glycosylation based on knowledge from cell systems [32]	☐ *Opportunities* — improved reactor design for synthesis reaction [34]
☐ *Threats* — advances in cost reduction of CHO platform	☐ *Threats — advances in cost reduction of CHO platform, lower costs in E. coli, nonmammalian PTMs*	☐ *Threats* — failure to reduce costs as technology advances

Abbreviations: ADC, antibody drug conjugates; CHO, Chinese hamster ovary cells; IP, intellectual property; PTM, post translational modification; BMC, bacterial micro-compartments.

number and molecular diversity will draw increased costs, as each will require a bespoke process and regulatory filing.

These restrictions may make their development uneconomic or severely limit patient access to such medicines based on cost. CFS has the potential to address all of these issues: the increased process agility and simplified production process, elimination of cell line development and prior knowledge of feed components to DSP, facilitates a streamlined approach to process development. A distributed production model where manufacture is performed close to the patient in small highly reproducible batches using CFS provides for a future where we can deal with stratified biological products with limited stability/shelf life. However, such a distributed production

Figure 2



Flow chart summarising the linkages between the factors that will determine the feasibility of using a cell free protein production system for the production of stratified medicines.

model may require changes to regulations be made before it can be widely adopted.

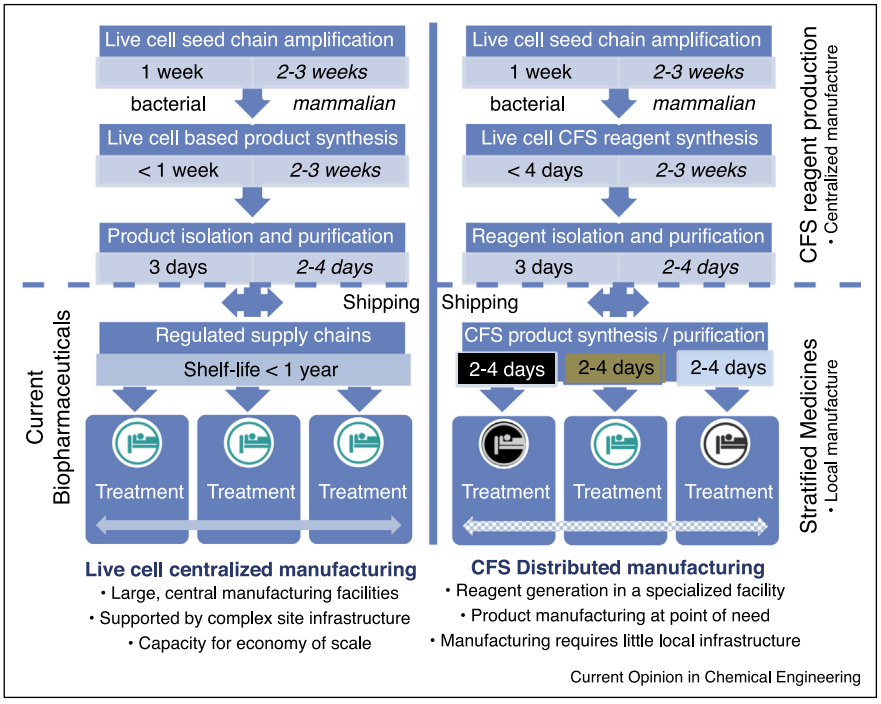
Why CFS for stratified medicine manufacturing?

Most of the uses of CFS to date have been in early stage research, screening and development, structural studies, or the production of novel therapeutic through the addition of non-natural amino acids in relatively short periods of time [18••,33]. In these examples the CFS value proposition has been process agility and high relative volumetric yields: the ability to rapidly produce small amounts of multiple different proteins with little process infrastructure. This process agility is a key differentiator if CFS is to become an alternative to live cell processes. But in order for CFS to progress in this direction several key factors around scale-up and manufacturability must be addressed. Figure 2 shows an analysis of a CFS as a bio therapeutic manufacturing technology identifying the critical process attributes (CPA) and describing how they interact to define a process that is fit for purpose. The four major areas of focus are Advantage, Scalability, Manufacturability, and Sustainability of the Supply Chain, which we shall now discuss.

Sustainability/supply chain

The sustainability/supply chain component is evaluated in comparison to a whole cell fermentation based process where the complexity of sustaining and monitoring a fermentation requires sophisticated and complex control systems and the supporting infrastructure, cell source amplification (master cell banks, production cell banks), as well as a well-developed supply chain that delivers the multiple components that make up the media used in a typical fermentation. In a CFS the generation of the protein production machinery (the ribosomal extract) is separated from the protein production (Figure 3) and is therefore essentially a well-defined chemical reaction whose major points of variation between different product types are the plasmid DNA content, thus simplifying the supply chain management for the manufacturing process.

Figure 3



Process overview for a distributed CFS system showing the advantages and disadvantages of different types of manufacturing model. Solid lines represent transfer of materials while dashed lines represent transfer of information.

Manufacturability

The direct competition for CFS is live cell fermentations. With durations of 2 days to 2 weeks and a potential for significant batch-to-batch product variation, live cell based processes require a significant investment in infrastructure (utilities, control systems, among others) to provide a robust manufacturing platform. CFS by contrast provide short reaction times, on the order of 4–12 hours, and because they are essentially chemical reactions can be designed to achieve high product homogeneity and little product batch-to-batch variation, which can have a positive impact on plant design in multiple ways.

Short reaction/fermentation times and batch-to-batch consistency creates an agile system where it is possible to combine multiple batches to supply market demand, to respond quickly with additional batches if patient demand increases, or to switch between products lines if necessary. The incorporation of single use systems into the production process further enhances agility. The agility of CFS is in contrast to live cell fermentations where batch-to-batch variations can make it difficult to combine multiple batches for a single product release.

Because of the short fermentation/reaction times, reproducible reaction/fermentations, and the ability to combine multiple batches for product release, producing a product in multiple small batches becomes technically feasible. The ultimate expression of this downsizing and process agility is a distributed CFS manufacturing model where production is conducted in small facilities at point-of-care (hospitals), and production is done in response to demand. One can envision a scenario where single batches are made for patient specific medicines and multiple small batches can be combined for treatments of rare diseases where scales are comparatively small.

Scalability

For any manufacturing technology scalability is of critical importance as most product development is done with material made at the small-scale. The ability to replicate the CQA of material produced in the lab at production scale is of critical importance if patient safety is to be maintained. As mentioned earlier cell free systems are capable of producing high specific product yields with low product micro-heterogeneity, but the high yields are often accompanied by an increase in process complexity which can have a direct impact on process scalability. Some of the more productive systems include the use of one or more of the following modifications: semi-continuous or continuous exchange bioreactors, regenerative energy systems, or more recently the use of microfluidic devices [34]. For example it has been shown that in CHO-based cell free protein synthesis the production of

antibodies requires the use of a semi-continuous format in order to obtain moderate product yields (>0.1 g/L) because of the depletion of critical components [33]. Recently Tran *et al.* [35] demonstrated the importance of measurement and control of the reactor environment to achieve >0.5 g/L product titres.

There are very few examples of CFS run at >1 litre scale. One such is the production of a cytokine at the 100 litre scale [36••]. This system uses an *E. coli* based ribosomal extract, engineered to promote protein folding and disulphide bond formation, and is run in batch mode. Published data indicates that this system produces high yields of active target protein (700 mg/L), is amenable to process optimisation using standard process optimisation tools (e.g. DoE, quality by design) and is scalable with consistent results obtained from 1 ml through to 100 litre scale operations. The company that published this data, Sutro Biopharma, is now moving towards the use of CFS for the manufacture and supply of their own novel biopharmaceuticals. In some cases, for example patient-specific or orphan disease treatments, larger batch sizes are not required. Timm *et al.* [34] have demonstrated a CFS microfluidic bioreactor model system for the production of single dose therapeutic proteins using a distributed manufacturing model. Using a dual channel reactor with an engineered nanoporous membrane between parallel reactor and feeder channels high product yield were achieved through the maintenance of adequate concentration of critical reaction components and the removal of inhibitory by products. With its small footprint, easily controlled CFS reactor and coupled/integrated downstream purification module, one could foresee an automated version of this system becoming the model point of care CFS system.

Concluding remarks

The demands of a changing pharmaceutical industry, responding to the opportunities of stratified medicine is leading companies to reflect as to the suitability of the current manufacturing models developed for a generation of blockbuster biopharmaceuticals. Cell-free synthesis (CFS) may offer a more agile platform better suited to distributed manufacture than cell-based systems. Though the technology continues to develop and mature from a renaissance of interest begun in laboratories of Jim Swartz at Stanford many questions and technological barriers remain. This review highlights:

- CFS has been successfully used to produce many classes of biotherapeutics though to this point not with a view to commercial manufacturing.
- Research and investment in CFS platforms based on *E. coli*, yeast and mammalian systems continue to grow.
- Use of CFS as a manufacturing platform for therapeutic proteins is in its infancy with Sutro Biopharma being the leading practitioner in this area. In addition to the use of CFS as a direct replacement for live cell fermentations academic groups are also investigating how CFS's other value propositions, that is, agility and simplicity can accelerate the acceptance of alternative manufacturing models such distributed/local manufacturing models which are more suitable for the production of stratified medicines.

The above indicates CFS has now matured sufficiently for it to be considered a viable candidate in the production of stratified medicines where its inherent process agility, process robustness, and product homogeneity are key value propositions. However, its widespread implementation faces several challenges including intellectual property rights, the high cost of the reagents, and sparse experience using the systems in a manufacturing environment.

Acknowledgements

Funding from the UK Engineering & Physical Sciences Research Council (EPSRC) for the Future Targeted Healthcare Manufacturing Hub is gratefully acknowledged (EP/P006485/1). Financial and in-kind support from the consortium of industrial users and sector organisations is also acknowledged. KMP acknowledges the support of EPSRC grant EP/K038648/1.

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