Therapeutic Protein Production: A Changing Landscape

K. John Morrow, PhD
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A Changing Landscape

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Executive Summary

Bioprocessing is the branch of biotechnology dealing with the production and purification of biological materials of commercial interest, mainly but not exclusively for the pharma industry. It is a wide-ranging discipline in which bioengineering, equipment design, molecular biology, cell genetics, cell culture technology, analytical chemistry, and polymer science are applied to the goal of rapidly, consistently and economically producing high-molecular weight, complex molecules.

In recent years there has been a huge expansion in the use of disposables at both the upstream and the downstream end of the production chain. Today, many GMP CMOs are available, and the customer has a vast range of options from which to choose. This has forced monumental changes in the industry, making available workable solutions at affordable cost to small companies and permitting the production of material for clinical trials of less promising, second-tier candidates. Many studies have been carried out comparing disposable versus reusable up/down stream technology. In almost every category—cost, speed of adoption and size of carbon footprint—disposable technologies far outperform reusables. The one exception is very large scale (several thousand liters and up), in which the large disposable bags are too cumbersome and hard to handle.

This exploding marketplace has caused major biotech companies to question their strategy of large capital investments in massive plants and reusable equipment. Indeed, some observers of the industry suggest that large biomanufacturing plants, gleaming with stainless steel, are akin to the dinosaurs and will never be replicated.

The upstream versus downstream gap continues to be a problem for the industry, given the fact that progress toward producing more protein per unit volume of culture medium has moved more rapidly than increases in the rates at which these materials can be purified at the downstream end. At the same time, there have been new developments in various biological systems, and a number of biologics manufactured with yeast, bacteria and plant systems are in clinical trials. These trends in both the upstream and downstream areas are encouraging, and should provide help in holding down the costs of these very expensive biological-molecule therapeutics.
While most antibody biologics are anti-cancer agents, there are a number of other areas of disease management in which biologics are starting to play a significant role. These include immune dysfunction, infectious disease and mental illness. Because of this expansion, there is a continuing demand for improved bioprocessing technology forcing the industry forward at a rapid pace. This expansion has motivated law-making bodies in Europe to redefine intellectual property guidelines and rules governing biogenerics. Legislation governing biosimilars has been passed in the United States as part of the recent 2010 healthcare legislation.

Much concern has been raised over the threat of Asian competition to the American bioprocessing industry. While the threat is cogent and serious, many analysts ignore a raft of problems that China must deal with as it struggles to move into high technology-based biomedical sciences. These include an oppressive political system, a large measure of discontent among the Chinese population, and a regulatory and intellectual property structure that will require years to mature. If China takes over high-tech bioprocessing as it has taken over the manufacture of Walmart items, it will be because the United States surrendered the industry without a struggle.

Management should also beware of aggressive competition from the European Union. With a combined population and GDP comparable or greater than that of the United States, and a mature regulatory and intellectual property framework, an excellent network of universities and research institutions, the European Union presents a formidable adversary. Throughout the continent, the construction of a robust 21st-century infrastructure is proceeding with a vengeance, promising to expedite the flowering of a high-tech industrial sector.

Medical device technology is advancing at a faster pace than drug development in general, and with increased cost savings and miniaturization, it is now feasible to install computer-controlled devices on disposable modules. With these new improvements in sensor technology coupled to advances in filtration technology, polymer chemistry and cell culture husbandry, the next decade promises to open up exciting changes in therapeutic protein production.
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An Industry Committed to Mammalian Cell Expression Systems (Golden 2009, See Appendix)

Due to the ease of manipulation, for many years bacteria were the agent of choice for the production of recombinant therapeutic molecules. Later, yeast and mammalian cells were widely adopted as improved vectors, host strains and a wealth of disposable cloning kits became available. In addition, as recombinant antibodies became viable therapeutic options, issues of glycosylation were addressed, causing mammalian cells to be widely adopted. Given the longstanding familiarity of the regulatory establishment with these systems and the reluctance of companies to risk delays and loss of profits, it is not surprising that the production advantage has remained centered on this triumvirate. Further, there are predictions that mammalian cells will remain in a lead position in the bioprocessing industry for the next decade. Wurm (as quoted by Morrow 2007) states: “We anticipate that mammalian cells will continue to dominate bioproduction for the next ten years, as new technologies carry the process forward.” However, there are indications that the situation may be changing as a wide range of species, including plants and various other choices, are coming under scrutiny (Table 1.1).

Table 1.1. A Wide Range of Species Under Study for Protein Production

<table>
<thead>
<tr>
<th>Platform</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammalian cells</td>
<td>Long-standing technology, glycosylation accurate, high levels of production, many FDA-approved products</td>
<td>Laborious construction of over-expressing strains; media costly; IP thicket</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Oldest approach, powerful for proteins without secondary modifications</td>
<td>Lack of post-translational modification, insufficient folding of proteins. Proteins tend to aggregate.</td>
</tr>
<tr>
<td>Yeast</td>
<td>Cheap to grow, extensive knowledge of genetics, vectors etc.</td>
<td>Post-translational modifications differ from mammalian systems; cell disruption difficult</td>
</tr>
<tr>
<td>Maize</td>
<td>Classical genetic target</td>
<td>Long developmental cycle</td>
</tr>
<tr>
<td>Duckweed</td>
<td>Growth media extremely simple, could be very economical</td>
<td>Regulatory history sparse</td>
</tr>
<tr>
<td>Microalgae (Carlier 2009, see appendix)</td>
<td>Potential for mega-industrial levels of productions (in the range of tons)</td>
<td>Low levels of synthesis; no regulatory history; more appropriate for industrial, non-pharma products</td>
</tr>
<tr>
<td>Insect cells</td>
<td>Eukaryotic, can carry out glycosylation, good for research-level quantities</td>
<td>Difficult scale up; low growth rates (Kovaleva 2009, see appendix)</td>
</tr>
<tr>
<td>Protozoa</td>
<td>Fully eukaryotic folding and modification; easily cultivated in vitro</td>
<td>No FDA products approved at this time</td>
</tr>
</tbody>
</table>

Source: Insight Pharma Reports
bovine-transmitted prions, could not be completely ruled out. Prions, small protein-based particles, have been known to cause a family of neurological disorders for many years, but only recently (Li et al. 2010) have been shown to undergo Darwinian evolution, continue to be a constant and significant threat to human and animal health.

These risks served as a powerful spur for companies to develop serum-free and protein-free culture media that would support mammalian cell growth on an industrial scale. While early studies by Ham and others laid the groundwork for a thorough understanding of mammalian cell nutritional requirements, only in recent years have media been developed that support the growth of CHO and other cells at the required levels for production performance. These studies are complex and laborious, and only applicable to a narrow range of conditions, given that even different clones of CHO may require different formulations for optimal growth (Butler 2005).

Media Development and the Evolution of Therapeutic Antibodies

As monoclonal antibodies have advanced through successive waves of technology dealing with exogenous proteins has been a constant issue in their development. As discussed by Grillberger et al. (2009), MAbs have moved from murine antibodies to chimeric murine/human antibodies and finally to fully human antibodies, with their manufacture increasingly dominated by the use of serum-free media. In parallel with these developments, has been the removal of animal proteins from all stages of their formulation. Prime examples are the tumor necrosis factor antagonists, the first of which was Remicade, a chimeric antibody grown in serum-containing media. It was followed by a fully human anti-TNFα (Humira) that is plasma-free, both in its manufacture and its formulation. The addition of serum proteins as excipients is being eliminated, as recombinant proteins are being developed to replace serum proteins (Jaber et al. 2007). Animal protein-free technology is being introduced to the production of vaccines, while egg-based technology is gradually being phased out in favor of recombinant proteins produced in serum-free, protein-free cell culture (Carpenter 2009).

With this background in mind, it is easy to appreciate why a wealth of technological innovations have been developed over the latter decades of the 20th century for optimizing mammalian cell expression. These investigations grew out of early efforts to understand mammalian cell nutritional requirements and deal with the challenges of maximizing production in cell lines. It should be noted that a number of difficult issues remain, including optimizing difficult molecules with a low protein yield, extensive post-translational modifications, and proteins with folding issues that can cause aggregation and protein malfunction. Nonetheless, an extensive range of therapeutic proteins are now produced in mammalian cell systems (see Table 2.1 below).
This range of new technologies opens many options for cell line optimization. Although regulatory risks and safety concerns are always an issue, these are being reduced as the industry gains experience in designer-built overproducers.

2.2. Mammalian Cell Culture Media

The second facet of the industry that enabled the huge productivity gains seen in recent years is the science of cell nutrition. Moving from basic investigations of metabolic pathways, many private companies have developed proprietary media to meet the demands of multiple grams per liter-producing cell lines. Biotech and pharma companies have contributed substantial resources to improving cell culture media, allowing them to focus on the metabolic processes and interdependencies that effect growth, viability and productivity.

Introduction to Mammalian Cell Culture Media Strategies

Given the preponderance of mammalian cells as the vehicle of choice for recombinant therapeutic protein production, it is not surprising that much effort has been expended in the optimization of their nutritional requirements. This work has a long history, going back long before the advent of the biotechnology industry. During the 1950s, media were formulated starting from an analysis of metabolites in animal sera. Pioneering studies in media development (Eagle 1965; Ham and McKeeham 1979) entailed optimization of nutrients one at a time, including carbon sources, buffers, amino acids and vitamins. Recently, there has been recognition that media design is a problem of the interaction of multiple variables, requiring a strategy that takes advantage of the automated technology now available in the biotechnology sector. Early investigations in cell culture nutrition utilized two-dimensional plots, testing cloning efficiency of a cell line as a function of concentration of growth factors one by one. However, since nutrients interact with one another, optimization requires multidimensional plots in which numerous metabolites can be adjusted simultaneously. As altering the concentration of one metabolite will doubtless affect the response of numerous other metabolites, this strategy allows a true optimization process to be realized.

Xcellerex (Marlborough, MA) has developed technology allowing these procedures to be carried out. Thousands of nutrient levels and combinations are run in parallel, achieving in one experiment what would take months to achieve in shaker flasks. This approach was used successfully to create an in-house, defined medium formulation for a cell line comparable in growth and productivity to the best commercially available proprietary growth medium formulation. It was also used to improve the feed formulation and strategy of an existing process, doubling the MAb productivity of a CHO cell line to 1 g/L. The technology was designed for increasing antibody production, but can be adapted for transfected cell lines producing other engineered proteins. The high-throughput approach gives the best chance of finding the production optima within time lines that are significantly shorter than companies typically spend on process development.

Elimination of Serum From Mammalian Cell Culture Media

From the early days of cell culture technology, animal serum was an essential component of practically all media formulations. As analytical techniques improved, it became evident that much of the need for serum was due to its ability to absorb trace toxins. In the 1970s, there was much concern over mycoplasma con-
Other Protein A Alternatives

A robust search for Protein A alternatives is in progress due to cost and other limitations (Baines et al. 2009). The main deficit of Protein A is its price, which for an industrial-scale antibody purification project can run to millions of dollars. Protein-based ligands are one alternative. To this end, Liu et al. (2009) have investigated an alternative to Protein A chromatography, a camelid antibody ligand. Members of the Camelidae family produce a native single-chain antibody consisting of a variable heavy-chain (VHH) region. The authors constructed a resin employing an anti-IgG single-chain camel antibody as its ligand. Although it did have a lower capacity than the leading agarose-based Protein A resin, it could be further optimized and demonstrated the additional advantages of stability in base and good host cell protein clearance. Although the new resin has been primarily marketed for IgG3 purification, Liu and his colleagues show that this product can potentially be used very effectively for industrial MAb purification.

Baines et al. (2009) state that while Protein A still remains the reagent of choice for full-length antibody purification, a new generation of synthetic ligands is under development, which will perform at the same level as Protein A, but possess improved stability and lowered cost.

Trends in Downstream Bioprocessing from the European Union

With the rise of the European Union in the last decade, member countries have vigorously pushed collaborative programs in many scientific areas. In the field of bioprocessing, the Framework for Downstream Initiative was established by the German BMBF (Bundesministerium für Bildung und Forschung; www.bmbf.bund.de) in conjunction with the German industrial and academic sectors. The BMBF plan of action will target development and evaluation of new methods to widen the existing field of separation techniques to achieve higher yields, purity and more favorable economics for biotechnical products. It will establish cooperative networks of the various players to optimize technology transfer. Finally, the program proposes the initiation of centers of excellence to nurture focused interdisciplinary working groups. The hierarchical framework will consist of young but experienced research scientists as leaders, selected from within Germany and abroad.
Are Revolutionary Developments Possible in Bioprocessing?

This report highlights that bioprocessing advances do not proceed by quantum leaps, but rather develop incrementally, with many small improvements that advance the industry forward. But there is one area which would revolutionize bioprocessing, that is, economical de novo protein synthesis, without the intervention of living systems. Currently, this is a very tall order. For instance, 4 mgs of a 200-amino acid peptide runs ca. $1,200/mg from a leading synthetic company, Genscript.

The company’s technology is known as FlexPeptide™, an approach to peptide synthesis based on a combination of automated synthesizers for liquid and solid-phase peptide synthesis, microwave technologies and ligation technology (Litovchick and Szostak 2008). The company claims this allows synthesis of demanding peptide sequences with success rates over 95 percent, including multi-cystine peptides with two disulfide bridges.

Genscript also uses microwave heating in automated peptide synthesis to decrease the time required to complete each cycle of coupling and deprotection. The microwave energy keeps the growing peptide chain from folding or forming aggregates and facilitates chemical bonding. These functions allow microwave energy to stitch peptides together to make longer peptides and to introduce various modifications to the peptide chains, including addition of sugars and phosphopeptides.

In addition to incorporated microwave technology, FlexPeptide™ employs proprietary ligation methods to build high yields of very long peptides by first synthesizing several shorter sequences and then ligating them together with high efficiency: 40–50 amino acids are routine and the platform can provide up to 200 without any technical modifications.

While the cost of these peptides is still prohibitive (currently in the range of $1B/kg!), the technology is developing rapidly, and it is conceivable that in the future this could be a feasible alternative to the cloning of a target gene and its engineering into a bacterial or mammalian cell host, along with the attendant complexities of expression, purification and polishing of a protein produced in a living system.

Disposables in the Ascendant

According to Roebers (IBC Bioprocessing Meeting, October 2009), the rise of biosimilars and the development of many new antibodies will inject more competition into the market and drive competition and the demand for reduced-scale capacity, as will the increasingly high titers achieved by the industry. Such trends will favor the small manufacturing facilities and the ever-increasing adoption of single-use technology. However, the monster dinosaur facilities have already been constructed, and while it is unlikely that any more such massive plants will be built, those already up and running will serve for manufacture of the “blockbuster” products in the range of 1,000s of kgs of material.
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### Therapeutic Protein Production: A Changing Landscape

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