PERSPECTIVES ON TECHNOLOGY TRENDS IN BIOPHARMACEUTICALS MANUFACTURE

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1. BIOPHARMACEUTICALS AND SOCIETY

Over the last 40 years, starting from being nowhere, Biopharmaceutical drugs have come to occupy a dominant presence in the list of top ten selling drugs globally. Today, worldwide biopharmaceutical revenues are estimated at USD 288 billion, or 23 % of global pharmaceutical industry revenues, and comprise seven of the top ten list of drugs (Table 1). Many of these drugs, particularly monoclonal antibodies, offer cures for difficult-to-treat diseases, mainly in cancer and rheumatoid arthritis.

However, these drugs cater only to approximately fifteen percent of global population, primarily because of affordability and accessibility issues. In order to expand the reach of these drugs, it is imperative for their prices to come down significantly. This can happen in two ways; expansion of biosimilars, which are to large proteins what generics are to small chemical molecules, and reduction in costs.

Till recently, the big biopharmaceutical manufacturers had little motivation to reduce costs, given that total costs of manufacture was a small proportion of the net selling price. With the emergence of cost-competitive, high-quality, biosimilar products with comparable safety and efficacy, big biopharmaceutical manufacturers would have to reduce price and therefore focus on reducing manufacturing costs to reduce impact on margins.

Governments in some developed countries have brought these products under the tender system and have therefore created another pressure point for a focus on manufacturing costs. Governments in many countries have also now placed biologics, and biosimilars in particular, as thrust areas for domestic production and self-reliance. Several policies, ranging from thrust area status, local manufacturing support, fast-track product registration, import duty benefits and reimbursements under health coverage to preference in government tenders, have been instituted. While these measures are undoubtedly important, manufacturing costs have to necessarily come down.

It is here that technology can play a big role, in addition to improving product quality, reducing time to market, scalability and better processing.

2. BIOPHARMACEUTICAL TECHNOLOGY

Small molecule drugs are inorganic and typically less than 900 Daltons (Da)in size. In contrast, large molecule biologics, such as proteins, are made up of several amino acids and are typically more than 1,500 Daltons (kDa) in size. (For reference one Dalton is equal to 1 atomic mass unit).

While small molecule drugs can be synthesised by a series of chemical reaction processes, proteins are far more complicated to make. The intermediate size molecules, between small molecules and proteins, are peptides and typically have 6 to 20 amino acids.

Proteins are made by engineering cells using recombinant DNA technology. This technology involves integrating genes that express proteins of interest into the genome of mammalian or microbial organisms. These engineered cell lines are grown in chemically defined media. As these cells grow, fed on oxygen, nutrients and vitamins in a medium, they produce proteins of interest; either within the cells or outside the cells. These proteins are harvested and purified. In monoclonal antibody technology, antibodies are generated by sensitizing cells to antigens. The genes of the antibodies are sequenced and used to create engineered cell lines by recombinant technology. These cell lines are used to produce monoclonal antibodies.

Cell lines can be microbial (e.g. *Escherichia coli*), mammalian (e.g. Chinese hamster ovary or CHO), yeast (e.g. *Pichia pastoris, Saccharomyces cerevisiae*) and insect cells (e.g. Sf9). One of the desirable characters of recombinant cell lines is to express product stably at higher volumes, such as 2,500Litres.

3. BIOPHARMACEUTICAL MANUFACTURE

Manufacture of biopharmaceuticals follows a process that can broadly be divided into four parts:

a) upstream cell culture in bioreactors or fermenters, depending on whether the protein is expressed in mammalian cells or microbial cells respectively.

b) downstream separation of expressed protein from within or outside the cells, depending on where the protein is expressed, by centrifugation or filtration.

c) purification, which involves a series of chromatographic steps, such as ion-exchange chromatography, gel filtration chromatography, as also other steps such as tangential flow filtration, ultrafiltration and nanofiltration

d) fill-finish for formulating and filling the proteins in single-dose vials, multi-dose vials, pre-filled syringes and cartridges,

These processes are carried out in clean rooms conforming to regulations and subject to rigorous audits. Typically, mammalian cell cultures grow slowly, while microbial cell cultures grow relatively faster. As a result, a batch of a mammalian cell culture process will take several weeks to harvest proteins from the cells, while microbial cell lines can grow in a few days' time.

4. TECHNOLOGY TRENDS

As with most technology sectors, biotechnology has also seen a rapid pace of development of new technologies.

5.1 Product Technologies

Science has come up with several new product domains in biopharmaceuticals.

Leading this facet of product delivery technologies is Small-interference Ribonucleic Acid (siRNA) or anti-sense technology. Another prominent development in this category are antibody drug conjugates (ADC) for the targeted delivery of anticancer drugs which are otherwise highly toxic, such as Kadcyla and Adcetris.

However the promise of this technology has not played out in practice. Issues around product safety for patients and long-term efficacy have resulted in their inability to meet targeted treatment outcomes.

5.2 Payload Delivery Technologies

Many biopharmaceutical products require delivery of proteins in a targeted manner to the diseased cells or tissues. This is analogous to monoclonal antibody technology. There have been several developments here such asdelivery of proteins into cells using virus-like particles (VLPs) and nano particle vehicles for intracellular protein delivery.

5.3 Ambient Temperature Protein Storage Technologies

Proteins are temperature sensitive, and have to be stored at temperatures in the range of 2 to 8^{o} C. This necessitates systems to continuously monitor storage temperatures; from manufacture to product administration into patients at the bedside. Excursions in temperature beyond the range mean non-compliance and naturally create product integrity questions. The cold chain infrastructure, continuous monitoring and periodic validation required, stability studies under different conditions and for different geographic zones collectively raise costs and management effort and intensity across the value chain.

Some technologies have evolved in making proteins thermo-stable, i.e. robust to wider temperature. These include formulations using novel excipients and encapsulating in

polymers. However, these technologies are far away from commercialisation, given that a long, capital-intensive process would be involved in proving their clinical safety and efficacy.

5.4 Product Expression Technologies

Improved expression systems with higher titers lead to reduced manufacturing capacity requirements. When it comes to higher levels of expression, several technologies are underway.

Chinese hamster ovary (CHO) cells are commonly used for the production of mammalian cell culture-based, recombinant therapeutic proteins. With increase in understanding of handling and engineering of Chinese hamster ovary (CHO) cells, expressions levels of upto 5gm/L have been achieved from 1 gm/L earlier.

At the same time, alternative expression systems are being developed. Avian lines (e.g., duck embryo quail sarcoma and chick embryo fibroblasts) have been reported to transfect well, have promoters that work with mammalian genes, and grow faster. These cell lines also promise higher levels of cell density and specific expression. Human cell line Per C6 has been proposed to be effective as well as beneficial for production of biopharma products.

Baculoviral insect cell systems have also been gaining popularity as a substitute for production of recombinant proteins and have been effective vectors for large-scale production of monoclonal antibodies.

Glycosylation plays a decisive role in ensuring proper protein function. Glyco Express is a novel expression platform that has emerged. This platform allows the generation of proteins with full human glycosylation and optimized sialylation. The technology is based on a glycol engineered human cell line and enables the production of large amounts of improved recombinant proteins. GlycoFi's technology is another novel technology used to control the glycosylation of recombinant therapeutic proteins.

5.5 Single Use Disposable Manufacturing Technologies

The focus on single-use or disposable systems in manufacturing increased with the need for facilities to produce multiple products in parallel. Single use can now be seen in laboratory, pilot, clinical, and commercial manufacturing operations. Single-use systems have been shown to reduce capital cost by 40–50%, reduce operating costs by 20–30%, and reduced time-to-build by 30% when compared with traditional stainless-steel technology.

Single-use technologies are becoming an industry standard for the manufacturing of clinical and production batches in biopharmaceuticals. They do away with high levels of capital expenditure upfront, give flexibility on operating scale and obviate large steam

requirements, cleaning-in-process and sterilization-in-process steps, thereby reducing product contamination issues. Above all, manufacturing facilities can be more compact with attendant benefits in capital costs or lease rents.

With their growing adoption, especially in clinical grade material and small volume product or country-specific manufacture, this technology is expected to find widespread use.

4.6 Protein Purification Technologies

Protein purification is an expensive proposition. Protein A is currently the most expensive chromatographic step. Some, lower-cost alternatives have emerged. These include precipitation, crystallization, cation exchange, and mixed-mode chromatography. Synthetic protein A resins are also available but have showed lower selectivity and affinity.

5.7 Microbial Contamination Detection Technologies

Rapid and reliable detection of microbial contamination is paramount for product safety. Conventionally, these tests are done manually using standard plate count methods. Rapid methods help reduce assay time using automated systems. High-throughput methods for detection of microbes will improve environmental control. Several new technologies in this area are emerging.

Bioluminescence, to measure light output produced by a reaction that is dependent on Adenosine Tri Phosphate (ATP) released from microbial cells, is one such technology. Flow cytometry, to detect microorganisms in liquid sample either in flow or captured on a solid surface by membrane filtration, is another.

5.8 Industrial Big Data Analytics

Just as with many industrial sectors, big data analytics have been making a beginning in biopharmaceuticals and have tremendous scope. Hybrid platforms are emerging, which are a combination of high-performance operational data management and data storage, in order to leverage technologies of real-time analytics. This promises to lead to designing of improved ligands and matrices, which allow shorter bioreactor residence times, higher flow rates and longer life cycles.

5.9 Robotics and Automation Technologies

Likewise, robotics and automation have started to make a mark in biopharmaceutical technology. High throughput robotics in both upstream and downstream process development can be vital to screen and improve cell lines, media compositions and chromatography media. High degree of automation degree and decreased scale (micro titer plates and tube bioreactors) will allow broader screens.

5.10 Continuous Process Manufacturing

Biopharmaceuticals are produced in batch processes or fed batch processes. With newer technologies, continuous manufacturing is becoming a possibility. This would encompass developments in perfusion bioreactors instead of fed-batch bioreactors, and smaller-scale modular chromatographic systems.

A series of small columns have been demonstrated to mimic one single large column with a diameter and a bed height equal to the total bed height of the smaller columns. Translated into practice, this could be imagined as an array of different types of columns, controlled by a microprocessor that assigns different types of chromatographic columns to different chromatographic cycle sequences; based on the manufacturing process specific for a product.

An analogy of this can be found in semi-conductor processing where machines specializing in a unit operation are assigned to carry them out for a bunch of materials, delivered to them by a materials handling and transport system; all controlled by microprocessors.

5.11 Process Intensification and Integration

With Process Analytical Technology (PAT) it is becoming feasible to merge characterization, release and online product quality assays. In addition techniques in modelling unit operations by Computational Fluid Dynamics (CFD) and M3C (modeling, monitoring, measurement, and control) of bioprocesses is emerging.

CFD is an engineering tool for virtual experiments and can predict fluid flow, heat and mass transfers. Results from CFD are relevant for a) process understanding, b) detailed product and process design, and c) conceptual study on cause and effect with a parametric operating conditions.

5. CONCLUSION

To conclude, it is fair to say that there have been no new radical technologies that have come about in the realm of expression of proteins; akin to recombinant technology and monoclonal antibody technology. However, there are several relatively less radical technologies coming into play in the near-term and medium-term that promise to change the face of the biopharmaceutical industry globally.

Collectively these technologies will impact the industry through better protein quality, higher expression of proteins in cells, higher yields per litre of bioreactor or fermenter volumes and higher levels of capture of proteins in the downstream separation and purification stages and better use of resources of biopharmaceuticals manufacture.

All this would translate into the desired outcome and imperative of lower costs of manufacture without compromising product quality. In turn, these technologies would have found a much deeper and intensive social engagement.

Table 1: LIST OF TOP-SELLING DRUGS WORLDWIDE

Sr. No.	Product	Pharma or Biopharma	Indication/ Segment	2014 Sales (USD billion)
1	Humira (Adalimumab)	Biopharma	Anti-inflammatory	12.54
2	Sovaldi (Sofosbuvir)	Pharma	Hepatitis C	10.28
3	Remicade (Infliximab)	Biopharma	Anti-inflammatory	9.24
4	Enbrel (Etanercept)	Biopharma	Anti-inflammatory	8.54
5	Lantus (Insulin glargine)	Biopharma	Diabetes	8.43
6	Rituxan (Rituximab)	Biopharma	Oncology	7.55
7	Avastin (Bevacizumab)	Biopharma	Oncology	7.02
8	Seretide/Advair (fluticasone+salmeterol)	Pharma	COPD	6.97
9	Herceptin (Trastuzumab)	Biopharma	Oncology	6.87
10	Januvia (Sitagliptin)	Pharma	Diabetes	6.00

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