

## Chapter Seven: **Scale-Up and Manufacturing**



The manufacturing of biologics is complex, since most are proteins—large molecules often variable in structure and sensitive to environmental conditions. The manufacturing of biologics has become a science that can be summarized in four key steps: producing the master cell line, growing cells and producing protein, isolating and purifying protein from cells, and preparing the biologic for patients. The whole process from creating the master cell bank to preparing the biologic for patients can take years and cost hundreds of millions of dollars.

## Using R&D Specifications

During the R&D phase, researchers develop the initial production methods on a small scale. They also determine the drug's final formulation, or physical form, for clinical trials—for a biotechnology medicine, usually an injection or infusion. Using all of the R&D data from these production steps, companies devise large-scale production methods to produce enough of the product for the intended market. The scale-up and manufacturing process must adhere to cGMP guidelines to ensure product safety and purity.

## Common Cell Lines

Many biotechnology products are proteins that must be produced by cells grown in culture. **Chinese hamster ovary (CHO) cells, nonsecreting (NS0) cells** (pronounced “NS zero”) and *E. coli* are cell lines used for production of biotherapeutics, especially monoclonal antibodies.

### BIOFACT



The American Type Culture Collection (ATCC) is a private, nonprofit resource dedicated to the collection, preservation and distribution of authentic cell lines and other biologic materials. ([www.atcc.org](http://www.atcc.org))

There are a number of reasons to use these cells. Both CHO and NS0 cells synthesize proteins much like human cells do. Both are **immortal cell lines**, meaning, they should be able to grow and produce product forever. Researchers are well versed in their optimal culture conditions. Both cell lines have **generally regarded as safe (GRAS)** status for therapeutic protein production. NS0 cells have the additional advantage of being programmed to produce antibodies, but they do not make or secrete any of their own antibody protein.

Other cell lines can be used and may be more suitable. The selection of the cell line depends on the expertise of the company, the properties of the cell and the regulatory requirements.

## Scale-Up Process

The scale-up of a cell culture process can be very difficult and time-consuming, taking as long as several months before researchers can obtain a product. The entire process of producing a biotech product from start to finish is often called a campaign and is usually divided into two main parts: **upstream** and **downstream**. Upstream processes involve production of the protein product, most often by using cells (microbial, insect or mammalian) growing in culture. Downstream processes include the recovery, purification, formulation and packaging of the protein product.

## Upstream Phase

Upstream processing begins with the cells that scientists create or engineer to make the protein product. Once the desired cell line is made, it is cryopreserved: scientists freeze a large number of vials of the cells to create a **cell bank**. To begin a campaign, scientists remove and thaw a vial of cells from the cell bank and initiate a cell culture in a flask containing a small volume of growth media. The initial volume of media can be as little as 5 mL. The media provides the nutrients and the optimum environment for cells to survive.

Scale-up is done by gradually transferring the growing cells into successively larger growth vessels containing larger media volumes. The cells are constantly dividing as long as the growth environment remains favorable. Therefore, more and more cells are present

## Cell Banks

Cell banks involve a two-tiered frozen cell banking system: a master cell bank (MCB) and a working cell bank (WCB). Scientists use one vial of cells from the MCB to create the WCB. Once established, the WCB is used to produce batches of product in the scale-up process. Working from the same stock of cell line reduces the chance of mutations associated with serial cultures.

The MCB is a reserve of cells that scientists use only when absolutely necessary. To protect the integrity of the cell lines, companies store their cell banks in two or more locations within their facilities and in one location off-site.

## Growth vessels vary in size:

- Flasks hold 5 mL
- Spinner flasks or roller bottles hold 50 to 200 mL
- Bench top bioreactors hold 5 to 20 liters
- Pilot scale bioreactors hold 50 to 200 liters
- Production vessels hold 20,000 liters or more

with each step. The greater the number of cells, the more protein product is generated.

### Scale-Up Monitoring

The goal of the scale-up process is to grow cells as quickly as possible and to produce as much protein product as possible.

Using the same assays or testing methods used in the initial R&D stages, scientists measure **cell viability** and concentration, product concentration and product activity at each incremental scale-up stage for monitoring purposes.

Lab technicians monitor and control the physical environment in which cell cultures grow. They do this manually in the initial scale-up steps to optimize growth parameters such as temperature, pH, nutrient concentration and oxygen level.

The monitoring process is automated once the cell culture is large enough to be grown in bioreactors.

It is crucial during the scale-up, **fermentation** and manufacturing stages that technicians monitor and test the cultures for contamination by bacteria, yeast or other microorganisms. Any contamination of a culture ruins the entire batch of product and costs a company money and time. Technicians follow very strict protocols for maintaining **aseptic** conditions at all times during the scale-up and manufacturing stages.

### Quality Control and Quality Assurance

Quality control (QC) and quality assurance (QA) departments are responsible for all of the monitoring that is crucial to the success of the scale-up and manufacturing stages of product development. The QC department

assures product quality and testing during the product development stages well before the product is at the stage of marketing, ensuring that the scale-up and manufacturing processes meet certain standards. The QA department is usually responsible for meeting and reporting quality objectives.

### Downstream Phase

In the **downstream phase** of manufacturing, the protein product is isolated from the cells that produced it. Proteins found inside the cell (**intracellular proteins**) require special protocols to extract them for purification. Usually this involves bursting the cells open to release the protein product, which then has to be purified away from the other components that were inside the cell. Proteins found outside of the cell (**extracellular proteins**) can be easier to isolate.

After harvesting the protein product, the next step is **clarification**. This is where scientists separate the protein from cellular debris. Then they apply the protein solution to a series of **chromatography** columns to obtain a pure protein product. Purification of protein mixtures by **column chromatography** separates proteins based on physical and chemical properties such as size, shape or charge (+ or -). Additional purification steps remove any residual DNA and deactivate any viral particles that may be present.

Researchers verify the isolation and purification of the protein product through confirmed testing protocols. The protein product is then formulated according to the R&D specifications and packaged for use by physicians and patients.