Erythropoietins, Erythropoietic Factors and Erythropoiesis
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Commercial production of recombinant erythropoietins

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Introduction

For the production of recombinant products from genetically engineered cells, a number of cell hosts may be used, but they generally belong to one of five categories: plant, bacterial, yeast, insect, or mammalian. Depending on the type of product that is desired, the features of that product, the intended use of the product, and the preferred method of manufacture, an appropriate host cell can be selected. With the appropriate genetic engineering, the gene of interest such as erythropoietin (EPO) can be produced by a host cell. In the case of EPO production, the sequence of amino acids, as well as the amount of glycosylation, must be correct to achieve the desired efficacy *in vivo*. This chapter describes one method of EPO production using cells genetically engineered to secrete recombinant human erythropoietin (rHuEPO). In this method of production, mammalian cells, which are capable of producing glycosylation forms with the desired efficacy in humans, are typically selected as hosts. The host mammalian cells secrete the rHuEPO product into the medium environment in which they are cultured, making the remainder of production a matter of separating the rHuEPO product from the cells and other components in the cell culture broth. This chapter summarizes the generation of rHuEPO-producing cell lines, the production of rHuEPO, the separation of rHuEPO from components of the cell culture broth, and the packaging of the final rHuEPO drug product.

Cell line development

The development of a cell line generates a consistent source of cells that is capable of satisfying commercial demand and that adheres to regulatory guidelines for genetically engineered cells. Development begins with the creation of genetically engineered cells that produce the desired product, and ends with a

cell bank, a homogeneous population of genetically engineered cells frozen at the same population doubling level.

Cell line selection and screening

After the initial genetic modification of a host cell population to produce the product of interest, an appropriate production cell line must be isolated and selected. Generally, populations of cells may be screened for desirable properties, such as productivity and the ability to secrete product of the desired quality. Products, such as rHuEPO, may be posttranslationally modified by the host cells (e.g., glycosylation), and these modifications can impact *in vivo* efficacy. Different populations of genetically modified cells may produce product with varying degrees of posttranslational modifications. Therefore, product quality can be an important endpoint when screening for an appropriate production cell line. Selecting the final production cell line may involve several stages of isolating (i.e., cloning) and screening candidate cell lines. To ensure a homogeneous production cell line, the final cell line will have typically been single-cell cloned.

Single-cell cloning

The industry standard for generating a homogenous population is single-cell cloning. Single-cell cloning is accomplished by placing one cell in a microculture environment, and then expanding that cell through increasingly larger cultures to generate a greater number of cells (e.g., 1×10^9 cells) (Fig. 1).

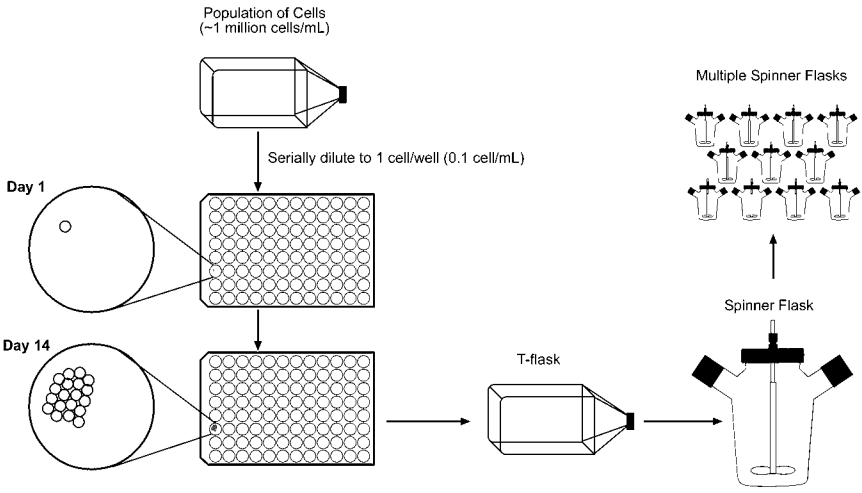

Figure 1. Single-cell cloning technique

Doubling of a single cell should produce two identical cells. Doubling again results in four cells that are assumed to be identical. Within a limited number of generations, expansion of the cells in this manner results in a homogenous culture of cells with identical genomes. When sufficient cells are amassed, cell banks may be created.

Cell bank

Once a cell line has been identified as the manufacturing line, a cell bank must be generated. The cell bank provides the starting material for all lots of product manufactured. For this reason, it is carefully scrutinized from both a business and regulatory perspective.

The master cell bank is the base starting material for the life cycle of the product. Cell banks are created in a two-tier system (Fig. 2). Each vial of master cell bank material can be used to create a working cell bank. Likewise, a single vial provides the starting material for a commercial cell culture lot.

Two-tier system: creation of master and working cell banks

A two-tiered approach is commonly used in industry to generate a supply of cell bank vials for the lifetime of a product. The master cell bank is generated first and typically consists of 100–400 vials. The master cell bank is not used by the manufacturing facility directly. Instead, the second-tier cell bank, the working cell bank, is used by the manufacturing facility. The working cell bank is generated by expanding one vial of material from the master cell bank. The working cell bank typically contains 300–1,000 vials.

Safety assessment

Once cell banks are generated, they must be characterized before they can be used to manufacture a drug for clinical use, as per regulatory guidelines [1–3]. Characterization is critical to ensure that the correct protein is being produced by the cells and that the cell line is not contaminated by adventitious agents.

The cell bank is the primary source for the recombinant protein produced, so verification that the correct molecule is being produced must be done at the inception of the bank. Confirmation of the DNA and/or RNA coding sequence ensures that the cells encode the required genetic sequence for the protein. Verification of the genetic sequence within the cell must be followed with verification of the amino acid sequence of the purified protein. Additionally, the cell bank must be examined to confirm that it is not producing any altered forms of the protein. For example, during gene integration in mammalian cells (after transfection or amplification), the cell's genome is rearranged, and

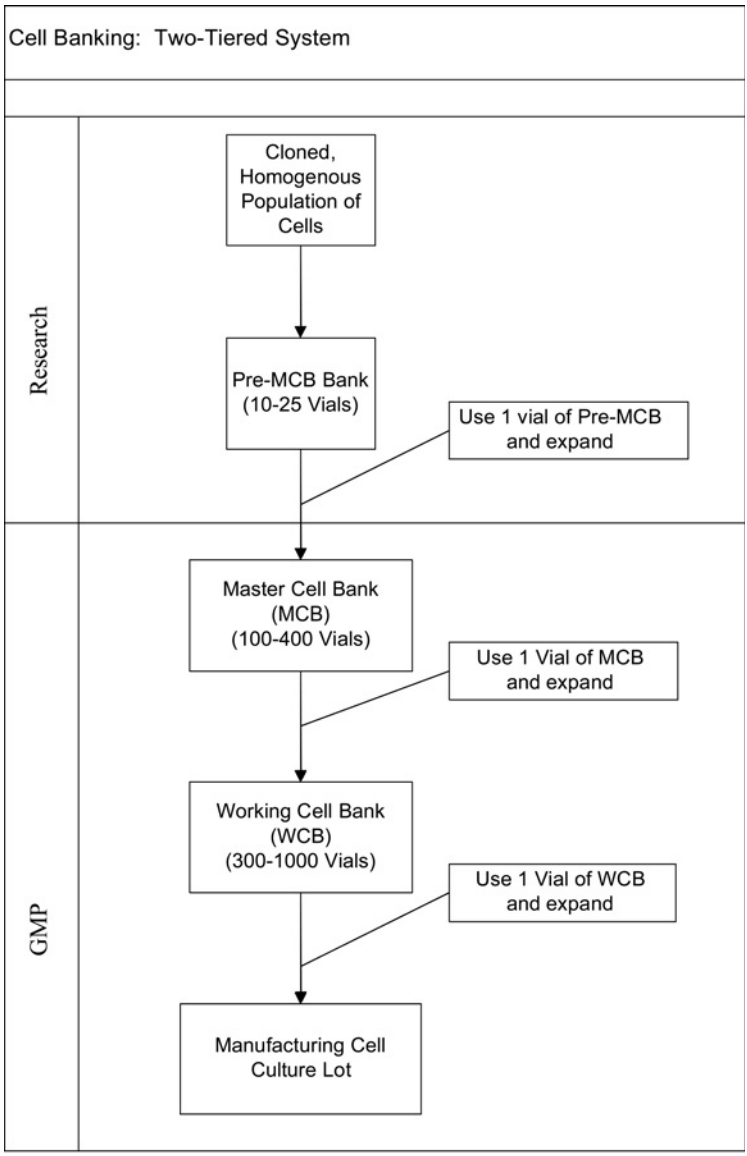

Figure 2. Cell banking is a two-tiered system. GMP = Good Manufacturing Processes; MCB = master cell bank; WCB = working cell bank

rearrangement possibly may involve the coding sequence of the protein of interest. One concern is that the rearrangement could occur at a site that results in a molecule containing a portion of the correct protein and a portion of another protein. In this event, the rearranged molecule could retain some of the correct protein's characteristics and be carried through a purification process;

however, the net result may be either the incorrect protein or a mixture of correct and incorrect proteins. Therefore, the cell bank is examined in great detail to ensure the absence of rearrangements that could lead to unwanted proteins being carried through manufacturing.

Ensuring that adventitious agents are not present is crucial. When a cell bank comprises mammalian cells, which theoretically can act as hosts to viruses, the cell bank is examined for mycoplasma, bacteria, fungi, and viruses. Numerous *in vitro* and *in vivo* assays are used to assess viral contamination. In the end, all of the aforementioned tests for adventitious agents must be negative. If they are not, the cell bank cannot used to make material intended for clinical use.

Cell bank stability assessment

The master cell bank is designed to last the lifetime of the product, so monitoring it and the working cell bank for storage stability is important. Cell banks are generally stored at –130 ºC or below to ensure cell stability. The cell banks are monitored by testing cells for viability upon thawing. If the cell bank's viability is stable, then no other testing is necessary. If viability is not stable, the bank is re-examined, and another master cell bank may need to be generated.

Cell culture process

One method of EPO manufacture uses cell lines genetically engineered to produce rHuEPO. In this chapter, an example of rHuEPO production in a cell culture process is presented, where cells from a mammalian cell bank are grown and product is secreted into the cell culture medium. The ability of the cell culture to produce product is affected by the nutrient environment and other physical parameters, such as temperature, pH, osmolality, and concentrations of dissolved gases. The cell culture process is designed to support the production of rHuEPO of a consistent quality, tailored to enable the product specifications and efficacy targeted by the manufacturer.

Raw materials

The raw materials for the cell culture process consist of the cell bank and the nutrients used to sustain and expand these cells. Nutrients in the cell culture medium generally are amino acids, additional carbon sources (e.g., glucose), vitamins, trace elements, growth factors, hormones, and salts. A review of media composition is given in [4]. Historically, cell culture medium also has contained serum from bovine sources, which may vary in composition depending on its source or the processing. Guidance for the industry for use of raw materials for the manufacture of biologicals is provided by CBER/CDER [1].

Process options

Many process options are available to produce a recombinant protein. One of the simplest cell culture options is batch mode, where the cells and the nutrient-containing media are added to a production vessel, and no further additions are made. Physical parameters (e.g., temperature, pH, and dissolved oxygen concentration) may be controlled within set ranges. The cells grow to the extent that can be supported by the initial nutrient concentrations; cells will stop growing and begin dying when nutrients become insufficient and/or if the amounts of waste products (the by-products of nutrient consumption by the cells) increase.

Fed-batch processes differ from batch processes by providing for the addition of nutrients into the culture as required. This method allows a much greater number of cells to be supported than batch processes. More cells typically translate to greater productivity due to the constitutive production feature engineered into most commercial cell lines. With constitutive production, as long as a cell is present and viable, it will continue to make product. Thus, the viable cell number correlates closely with the amount of recombinant protein produced.

In addition to supplementing a culture with nutrients as required, spent medium in the culture may be removed to reduce the amount of waste products. The process of feeding fresh nutrients and concurrently removing spent media is called perfusion. The spent medium, called the harvest, contains the protein product; the device used to separate the spent medium from the cells is called a cell retention device.

Operationally, batch and fed-batch processes are relatively easy to execute and perform reliably; perfusion processes are relatively more complex to exe-

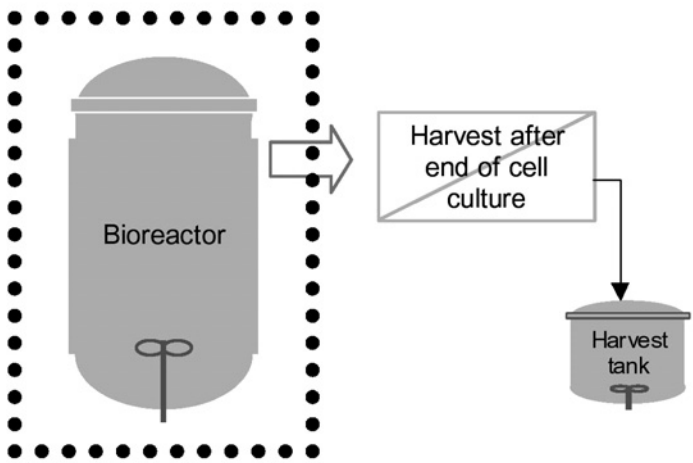

Figure 3. Schematic of a bioreactor operating in batch mode. The sterile envelope, depicted by the dotted line, encompasses only the bioreactor. The harvest operation, which includes cell separation from the conditioned medium and concentration of the conditioned medium, is not performed under sterile conditions

cute and in general use longer production times. The numbers of pieces of equipment that must be operated in a sterile manner within the sterile envelope are depicted in Figure 3, Figure 4, and Figure 5. The number of pieces and complexity of equipment in the sterile envelope correlates with the complexity in executing the three types of processes. A schema of the equipment (bioreactor, media feed tank, and harvest tank) used for each of these process options and the relative scale of tanks and production vessels is depicted. For batch and fed-batch processes, the production vessel is typically the largest piece of

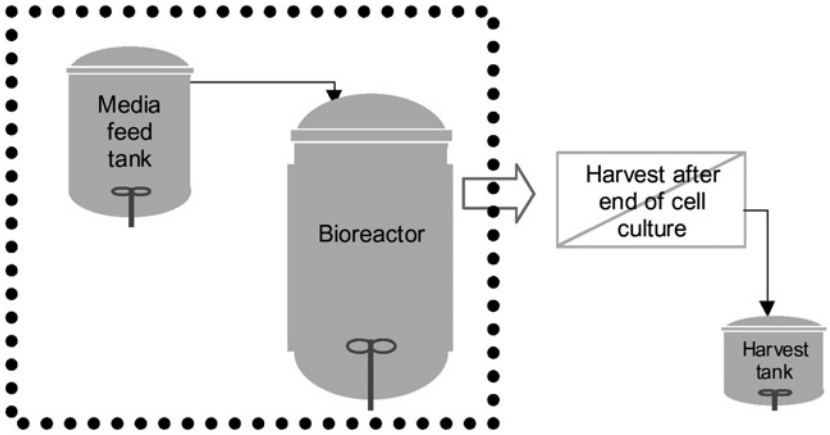

Figure 4. Schematic of a bioreactor operating in fed-batch mode. The sterile envelope, depicted by the dotted line, includes the media tank and the bioreactor. The harvest operation, which includes cell separation from the conditioned medium and concentration of the conditioned medium, is not performed under sterile conditions

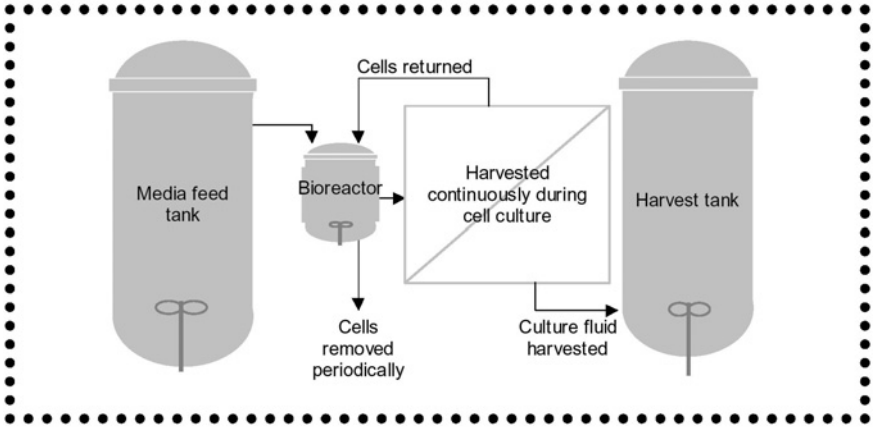

Figure 5. Schematic of a bioreactor operating in perfusion mode. The sterile envelope, depicted by the dotted line, includes the media tank, bioreactor, cell separation device (harvest unit), and harvest tank. The harvest operation here does not include any concentration of the conditioned medium.

equipment used in the cell culture process; for perfusion processes, the feed and harvest tanks are typically the largest equipment used.

The size of the equipment and the time over which each piece of equipment needs to be occupied for a given production run will influence the design of a manufacturing facility and the number of production runs a manufacturing plant can execute. The manufacturing facility is designed to comply with regulatory guidelines for GMP manufacture (i.e., CFR parts 210 and 211 are used in the US; guidelines differ slightly in other countries). Additional information on process options is available [5, 6].

Commercial manufacture

A commercial cell culture manufacturing process consists of a seed train followed by a production step. The purpose of the seed train is to generate a sufficient number of cells with which to initiate production in the production vessel. The required process conditions are imposed on a production vessel to generate optimal quantity and quality of product.

Seed train

A seed train describes the expansion of cells from a frozen cell bank vial to the inoculum of the production reactor. The volumes involved may range from 1 mL to 10,000 L. The vessels involved may include t-flasks, shaker flasks, roller bottles, disposable bag bioreactors and stirred tank reactors (Fig. 6). The reactors may be operated in batch, fed-batch, or perfusion modes. The purpose of each step of the seed train is to generate a sufficient number of cells of known quality to enable inoculation of the subsequent step. The culmination of the seed train occurs at inoculation of the production reactor.

Given a cell bank vial containing 1×10^7 viable cells, the initial seed train time to the production reactor may be estimated. First, the doubling time for the cell line must be established. For mammalian cells, doubling time typically ranges from 16–48 h. Second, the requirements for cell number generated at the end of the seed train must be established. For example, if the process uses a 10,000 L production reactor and the production reactor requires an inoculum density of 1×10^6 viable cells/mL and an initial volume of 7,500 L, then 7.5×10^{12} viable cells are required. The number of cell doublings (n, or population doubling level) from vial thaw to production reactor inoculation can be given by the formula:

$$n = LN(XS / X0) / LN(2)$$
$$n = LN(7.5 \times 10^{12}/1 \times 10^7) / LN(2)$$
$$n = 19.5$$
$$n \sim 20$$

where LN = natural logarithm and XS final cell number = 7.5×10^{12} and X0 = initial cell number = 1×10^7

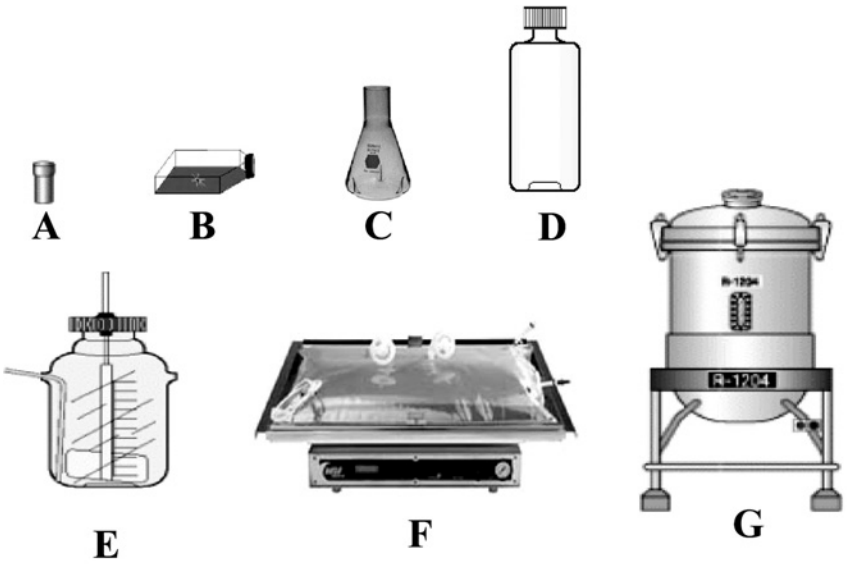

Figure 6. Exemplary vessels used in the preparation of a seed train: (A) freezer vial, (B) T-flask, (C) shaker flask, (D) roller bottle, (E) spinner flask, (F) Cellbag, (G) stirred tank reactor

Using a conservative estimate of 2 days for the doubling time (td), the overall seed train time is $n \times td = 20 \times 2$ days = 40 days, or approximately 6 weeks.

Multiple seed trains using culture taken from an existing seed train may be initiated if cell numbers allow and if the cell line's productivity remains stable. Thus, multiple production reactors stemming from a single cell bank vial may be considered to shorten the overall time to inoculate production reactors. Alternatively each production reactor may be derived from a single vial thaw.

Production

Once the seed train has generated a sufficient number of cells from the cell bank, these cells can produce rHuEPO. Cells can adhere to a solid substrate and secrete rHuEPO into the liquid cell culture medium. Examples of solid substrates include tissue culture plastic such as roller bottles or microcarrier beads that may be made of ceramic or other porous materials. Alternatively, cells can be freely suspended in liquid medium where they secrete rHuEPO. Exemplary cultures in roller bottles and tank vessels are shown in Figure 7 and Figure 8, respectively.

Commercial cell lines are designed and selected for constitutive production; therefore, the amount of rHuEPO produced correlates with the viable cell number (Fig. 9). For adherent culture, the number of cells is limited by surface area. To increase production of rHuEPO, the surface area is increased, typically by increasing the number of roller bottles or microcarrier beads. Since nutrients are supplied in the liquid phase and products are secreted (by the cells) from the

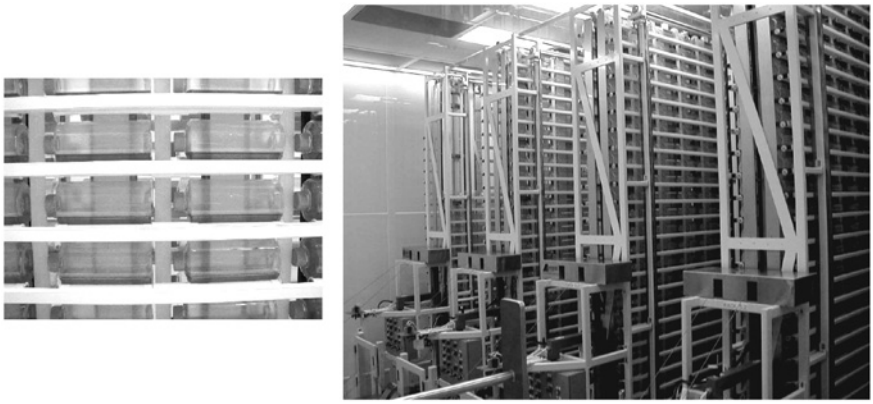

Figure 7. Production of rHuEPO in roller bottles (left panel). The right panel shows large racks containing many of the same type of roller bottle cultures.

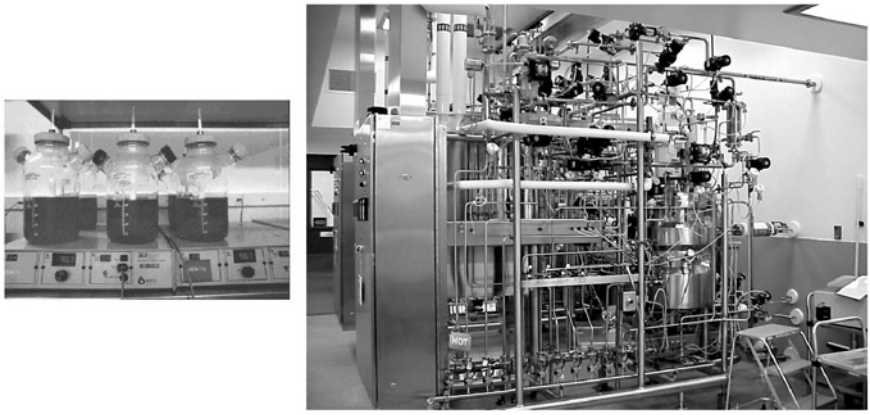

Figure 8. Production of rHuEPO in tank vessels. A medium containing rHuEPO-producing cells is shown in the spinners on the left. The stirred tank reactor shown on the right also contains a culture of rHuEPO-producing cells.

solid phase into the liquid phase, the successful mass transfer of nutrients to the cells, and rHuEPO and metabolic waste products from the cells must be carefully considered in the design of the process. A commercial production lot from adherent cultures with roller bottles typically entails using many small production vessels; the challenge of such a process for commercial scale production is to demonstrate equivalent control (i.e., monitoring) of each of the multiple production vessels. The degree of control required is imposed to achieve comparable growth and productivity performance from each production vessel. After product is produced, it is separated from the cells in the harvest process. Since cells are attached to the solid substrate, harvesting the rHuEPO product in the

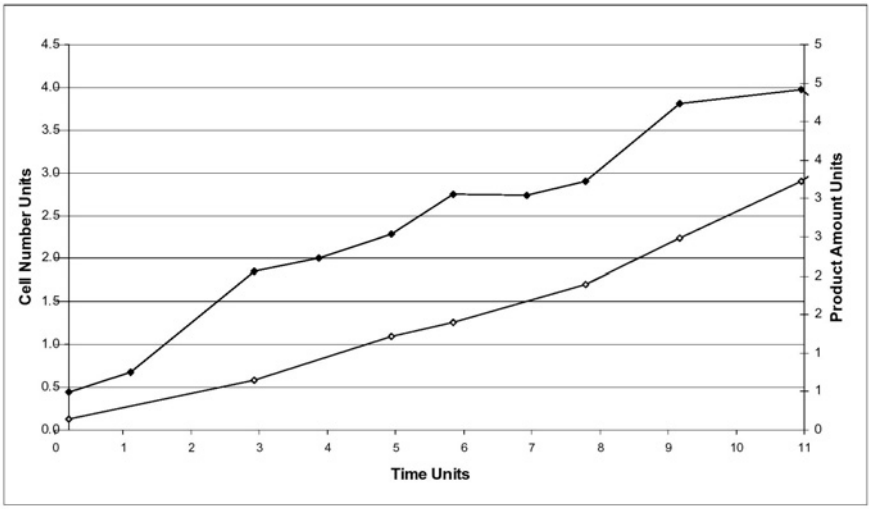

Figure 9. Correlation between number of cells and amount of product produced. Solid diamonds = cell number profile; hollow diamonds = product amount profile.

liquid conditioned medium is operationally straightforward and involves pouring off or decanting the liquid before volume reduction and purification.

For suspension cultures, scale-up is more straightforward. To increase production, the number of cells per unit volume (i.e., cell density) may be maximized, and/or the volume of culture may be maximized. To maximize the cell density, limiting nutrients within the culture environment are identified and supplements are added to the culture. The medium is sufficiently enriched to support the maximal number of cells. To maximize the culture volume, a larger scale of production vessel may be used. Both cells and product are distributed homogeneously in well-stirred reactors so that the growth and production performance within each volume of liquid is consistent. The volume of liquid may be increased as long as homogeneity is achieved (i.e., mixing is adequate). Stirred tank reactors for suspension cultures of mammalian cells typically range between 1,000 L and 20,000 L. To harvest the product, the cells must be separated from the liquid medium. Separation is achieved by microfiltration or centrifugation. The clarified liquid-containing product may then be concentrated for volume reduction and purification.

Within any of the process modes described, a cell line that has been engineered to produce rHuEPO may grow and secrete product encoded by the *EPO* gene. Not all of the secreted product may qualify as the rHuEPO sold by a manufacturer. The EPO molecule has 3 *N*-glycosylation sites and 1 *O*-glycosylation site, leading to the possibility of differently charged molecules with different isoelectric points (i.e., isoforms). Those molecules with the lowest isoelectric points, typically corresponding to those having the greatest amount of sialylation and glycosylation branching, have the greatest *in vivo* efficacy.

Molecules with higher isoelectric points, or less sialylation and less glycosylation, have less *in vivo* efficacy, and can be removed in purification. Additionally, even if the cell produces product of the desired charge profile, this product may be degraded in culture by proteases. Further, the product may form undesired complexes such as aggregates, which must be separated from the desired monomer. The cell may be influenced to produce increasing amounts of the desired charge profile by controlling process conditions, such as temperature, pH, osmolality, and nutrient and waste product concentrations. Similarly, undesired forms of the product may also be minimized.

Recovery and purification of erythropoietin

A number of purity criteria must be met for rHuEPO to be a viable human pharmaceutical. Host cell and other contaminants, such as DNA, host cell proteins, and endotoxin, must be removed to appropriate levels. The removal or inactivation of any theoretical endogenous or adventitious viruses must be shown. Amounts of product-related contaminants, such as aggregates, proteolytically degraded, underglycosylated, or oxidized forms, are minimized. A targeted purity is specified by the manufacturer, so each product lot must meet the criteria described in the manufacturer's product specification. Product specifications may differ among manufacturers.

The clearance rate of rHuEPO in the human body is affected by the number of sialic acid residues on the carbohydrate portion of the molecule. Under-sialylated rHuEPO is cleared in the liver by the asialoglycoprotein receptor [7]. Selecting for molecules with the greatest degree of sialylation gives a product with the desired pharmacokinetic properties, which affects the biological potency of rHuEPO. Different manufacturers may have different selection criteria (and therefore different product quality characteristics) for the EPO molecules that are retained in the recovery and purification processes.

In addition to having a well-understood and reproducible recovery process, a number of regulatory requirements exist for the manufacture of biopharmaceuticals. The product must be produced under Good Manufacturing Practices (GMP), which encompasses a range of topics from plant design, which affects air and water quality, solvent usage, and waste disposal, to day-to-day operations, which include operator training, batch record writing and review, quality assurance, and cleaning validation. Many of these guidelines can be found in Q7A 'Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients' [8].

Concentration and diafiltration

After the clarified cell culture media is harvested, it must be concentrated, and the buffer salts and other high conductivity/low-molecular-weight components

must be removed. Concentration is an important step, since it is difficult to store and handle large pool volumes. The expense of storing large volumes of harvest media in a frozen state can be prohibitive. In addition, the time required to load a large volume of media on to a column decreases plant productivity. Prolonged column load times can also lead to proteolytic degradation of product in the harvest media as it awaits loading onto the column. Removal of buffer salts and other high-conductivity, low-molecular-weight components is important because these may interfere with binding to the purification columns. For example, ion-exchange chromatography depends on ionic interactions between charged groups on the protein and oppositely charged groups on the chromatography media. The high conductivity of the harvest media would prevent protein from binding to the ion-exchange chromatography media.

Concentration and diafiltration are done using ultrafiltration membranes. This operation allows for the passage of low-molecular-weight solutes and water through membrane pores and the retention of larger-molecular-weight solutes, such as rHuEPO and other proteins. The driving force for the passage of these solutes and water through the pores is the pressure difference across the membrane. The membranes can be made of cellulose, polyether sulfone, or other polymers. Membranes are available that have different 'nominal molecular-weight cut-offs', ranging in molecular weight from 5,000 to 500,000. Proteins and other high-molecular-weight materials that do not pass through the membranes are referred to as the retentate; the material that passes through the membranes is called permeate. The rate at which the water and solutes go through the membrane is called the permeate flux rate and is usually measured in litres per minute.

During the concentration phase (Fig. 10a), both water and solutes are forced through the pores, leading to a volume reduction in the harvest medium. The

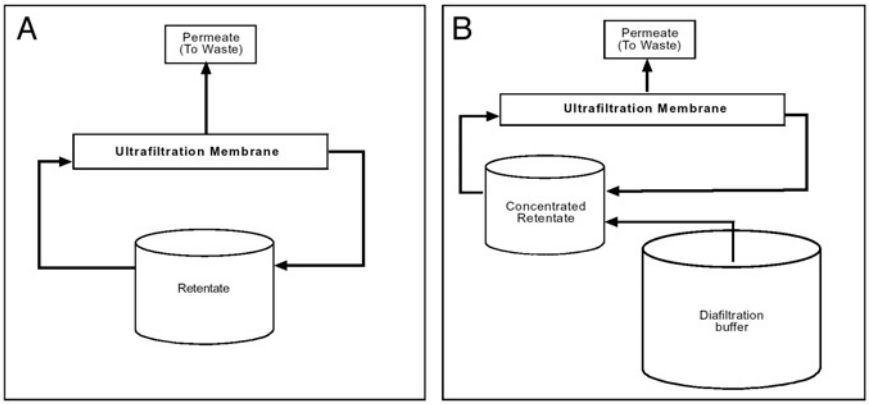

Figure 10. Panel A – Concentration mode. Clarified cell culture medium volume is reduced in retentate to make concentrated retentate. Panel B – Diafiltration (DFM) mode. Removal of high-conductivity components in concentrated retentate to make DFM.

range of this volume reduction may be 5- to 100-fold depending on what is required for a given protein. During the diafiltration phase, the concentrated harvest continues to be fed through the membrane; at the same time, however, a lower conductivity buffer (diafiltration buffer) is pumped into the harvest media retentate at approximately the same rate as the permeate flux rate (Fig. 10b). This process causes a buffer exchange of the original high conductivity buffer for the lower one. The greater the number of retentate volumes pumped though the membrane during the diafiltration step, the more complete the buffer exchange. This product pool is referred to as diafiltered media, which either can be immediately processed through the subsequent purification steps or stored frozen and processed later.

Chromatography: isoform selection

An anion-exchange media can be used to separate molecules on the basis of molecular charge. This chromatography may be used to separate rHuEPO from host cell proteins, nucleic acids, and endotoxin.

Chromatography: removal of contaminant proteins

A reversed-phase chromatography step can be used to remove host cell proteins and additional nucleic acids. The interactions of the column matrix with proteins are mostly hydrophobic in nature. Once bound to the column, a protein can be eluted with another hydrophobic, low-dielectric organic solvent. The protein portion of rHuEPO is quite hydrophobic because of the large proportion of hydrophobic residues and its solubility characteristics after removal of the polysaccharide portion of the molecule. Gel filtration chromatography, also known as size-exclusion chromatography or molecular sieving, may also be used to separate a protein mixture based on molecular weight. The media consists of agarose or acrylic beads of defined pore sizes. Proteins too large to penetrate the pores are excluded from the interior volume of the beads and have a shorter elution path through the column (hence they elute sooner). Smaller proteins either partially or completely penetrate the pores, resulting in a longer elution path and longer elution times.

Viral clearance

If rHuEPO is made in mammalian cells, the ability to remove theoretical endogenous or adventitious viruses from the product must be shown. Because virus titers are well below the level of detection, bench-scale studies are done in which different types of model viruses are deliberately added to process streams to demonstrate the ability of the process to remove viruses. Usually

several types of viruses are used, including enveloped and nonenveloped viruses.

Dosage form (drug product) manufacturing

In general, dosage form manufacturing of rHuEPO, as with all recombinant protein products, is governed by national governmental regulatory agencies to ensure that biologic production facilities adhere to sound quality control and current GMP. Facilities that produce rHuEPO are routinely audited and inspected to ensure compliance and patient safety. In the United States, biologic production for parenteral applications is governed by the Food and Drug Administration (FDA CBER/CDER); regulations are covered under section 21 of the Code of Federal Regulations Parts 210 and 211. These regulations differ slightly from country to country. All manufacturing plants and processes are validated and filed with the FDA (or regional authority) before marketing approval is given. All operations are conducted in an aseptic, temperature-controlled, highly monitored environment, with final filling operations done under conditions that typically allow only 100 particle counts/cubic foot of air in the filling operation suite. For dosage form/drug product manufacturing, maintaining validated processes, quality control, and aseptic conditions are critical to patient safety and product integrity.

In addition to regulatory compliance, a primary concern for recombinant proteins, and in particular rHuEPO, is the maintenance of product integrity by minimizing physical or chemical degradation. Product integrity is maintained from the bulk stage through the final dosage form by adjusting process parameters and the composition of the final formulation. To mitigate process impacts, rHuEPO is formulated in solutions that often contain human serum albumin or polysorbate that acts as a protector against surface adsorption, surface denaturization, shear forces, chemical degradants, and other deleterious factors. Because of the relatively low concentrations of rHuEPO used clinically, additives are important to protect the product during processing. Formulations are optimized for stability and parenteral delivery. Ingredients such as citrates, chlorides, and phosphates at relatively neutral pH are often used. Nonoptimized formulations can result in degradants that arise before the end of shelf life. The formation of degradants can lead to loss of activity or byproducts that may potentially be antigenic.

rHuEPO is marketed in a variety of formulations and concentrations, providing the practitioner with a wide selection of dosages for achieving optimal hematocrit values for patients. The final dosage form available in clinics is typically an aqueous formulation in a vial. Prefilled syringes, lyophilization, frozen liquid, or other delivery forms also can be used. This variety of formats can complicate production because of the need to formulate at different strengths, different fill volumes, and different delivery vehicles.

Process

Typical processing of rHuEPO consists of several unit operations: buffer preparation, formulation of the purified bulk, filtration of the formulated product, filling into a delivery vehicle, lyophylization (i.e., freeze drying if applicable), unit inspection, and packaging. Figure 11 depicts a generic process flow from bulk though packaging operations. A formulation-and-fill process may comprise several vessels, a fill line, and inspection packaging lines. One vessel may be used for buffer preparation, another for active addition to the buffer, and a third for holding the final formulated product before filling. The set up can vary depending on batch size and the formulation being produced.

General processing conditions must be closely examined to ensure product compatibility. Typically, the following items must be examined: materials in the processing vessels and transfer lines; duration of product exposure to various temperature and sterility conditions; and shear forces associated with mixing, filtration, and filling. During the formulation-and-filling operation, rHuEPO primarily comes in contact with stainless steel. Exposure to silicone tubing, various filtration membranes, ceramics, and glass can occur, however. Generally, process conditions are tested, and the product is placed on a stability testing protocol to ensure that no deleterious effects result from processing.

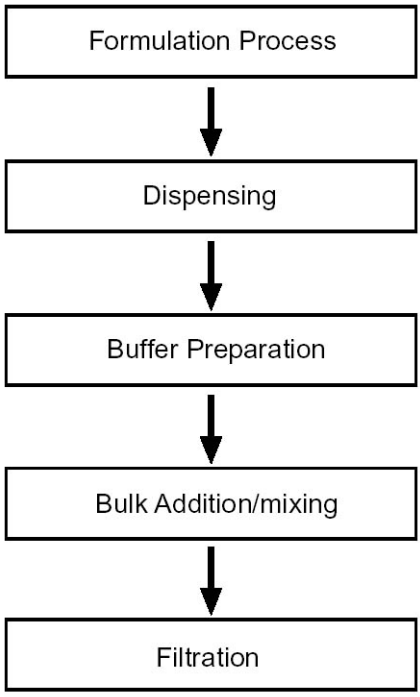

Figure 11. Generic process flow from bulk to packaging operations

The initial step of the dosage form manufacturing is bulk dispensing and addition of materials associated with the final formulated product. Product amounts are adjusted according to batch size and concentration (potency). Additional excipient raw materials are added before production. Before use, each material is examined to ensure that safety, quality standards, and product-formula requirements are met. Buffer preparation is completed by the addition of the excipients to purified Water For Injection in a processing vessel. The buffer can be filtered to clarify and to remove particulates. After buffer preparation, the bulk rHuEPO is added. Additional components that protect or stabilize the rHuEPO can also be added. The formulations or stockkeeping units that are used in multidose settings will contain an antibacterial agent to minimize microbial growth after initial breech of the stopper septum. The bulk rHuEPO product together with the excipients added to make up the formulation is referred to as the formulated bulk.

The formulated bulk is sterile filtered into a holding vessel and stored until filled. Dosage-form components such as vials or syringes are filled using automated equipment in a clean-room environment. The filled vials and syringes are immediately sealed with a stopper, and capped (in the case of vials). Product contact components such as stoppers, vials, and syringes are washed and depyrogenated at high temperature or sterilized in the presence of steam. Some components can be purchased from vendors pre-sterilized and ready to use.

Each filled vial or syringe should be inspected for particulate matter in solution or cosmetic defects, such as marks or scratches. Additionally, each unit is inspected to ensure that proper lot number and expiration date have been applied to the label.

Release

Before the release of any rHuEPO lot, the final material is thoroughly tested to ensure sterility, concentration, lack of endotoxin and bioburden, and product integrity according to the manufacturer's quality control requirements. Additionally, batch records from the manufacturing run are reviewed to ensure that no deviation was made during the production run. Finally, as each lot is released, the product is shipped to wholesalers according to validated procedures.

Summary

An example of commercial production of rHuEPO is discussed in this chapter, where the manufacture starts with a cell line that is engineered to produce product in commercial quantities of required quality. The cell line is banked, and the cell bank is used as the starting material for the manufacturing process. In this example, rHuEPO is secreted from genetically engineered mammalian

cells in a cell culture process and then recovered and purified as rHuEPO bulk in a purification process, to achieve the desired product characteristics specified by the manufacturer. Product from different manufacturers may have different quality characteristics. The rHuEPO bulk drug substance is formulated to achieve the required dosage forms for the final drug product. The manufacturing process is performed in a cGMP facility and monitored for consistent performance. Regulatory and other safety requirements are followed to reproducibly produce a safe and efficacious product.

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