CELL CULTURE METHOD

FIELD OF THE INVENTION

[0001] The present disclosure is in the field of cell culture methods for producing therapeutic proteins.

CROSS-REFERENCE TO RELATED APPLICATION AND INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0002] This application claims the benefit of priority to U.S. Provisional Patent Application No. 63/129,349, filed December 22, 2020, which is hereby incorporated by reference in its entirety.

[0003] Incorporated by reference in its entirety is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: ASCII (text) file named "55498_Seqlisting.txt", 4,255 bytes created December 21, 2021.

BACKGROUND

[0004] Clinical manufacture of therapeutic proteins is an expensive, large scale endeavor. The production of recombinant polypeptides for use as pharmaceutical therapeutics typically utilizes a drug substance cell culture process that consists of a series of scale-up and expansion phases designed to generate sufficient cell mass for inoculation of a production bioreactor. The cell culture process is initiated by thawing a vial from the working cell bank (WCB) and expanding the culture by using, for example, a series of shake flasks, culture bags, and/or expansion seed bioreactors (e.g., N-3, N-2, wherein the number indicates how many steps antecedent a bioreactor is from N, the final, or production, bioreactor), or the like. Following growth in the seed culture bioreactors, the culture is transferred to an N-1 bioreactor, which can be, for example, a perfusion bioreactor. In an N-1 perfusion bioreactor, the culture is perfused with fresh medium in order to generate sufficient cell densities for inoculation of the final cultivation step, the production bioreactor (N). The production bioreactor is operated to maximize the efficient production of the recombinant polypeptide.

[0005] Most of the efforts to date to improve the efficient production of recombinant polypeptides have involved manipulating the N production bioreactor parameters. Methods and systems used in the scale-up and expansion phases remain complex, labor intensive, cumbersome, and present multiple challenges in expanding therapeutic protein production facilities. Yet, alternative methods and systems for scale-up and expansion phases have proven elusive because of adverse effects on cell titer and culture viability for N production runs, as well as inconsistency in the final therapeutic protein product. As the demand for greater quantities of therapeutic recombinant proteins increases, there is a need for alternative methods and systems for cell expansion and recombinant protein production which minimizes the disadvantages of current protocols.

SUMMARY

[0006] The disclosure provides a method of producing etanercept from Chinese hamster ovary cells (CHO). The method comprises running an N-1 bioreactor system using a recirculating tangential flow filtration (RTF) or alternating tangential flow filtration (ATF) cell retention device under conditions that maintain a cell aggregate size of at least about 20 μm (and, optionally, not greater than about 200 μm; e.g., between about 20 μm and about 150 μm or between about 20 μm and about 95 μm) before running an N production bioreactor. In one aspect, the present disclosure relates to a method of producing etanercept from Chinese hamster ovary cells (CHO), the method comprising running an N-1 bioreactor system using a recirculating tangential flow filtration (RTF) or an alternating tangential flow filtration (ATF) cell retention device under

conditions that maintain a cell aggregate size of at least 20 μm. In various examples, these conditions provide an η of at least about 20 μm (and, optionally, not greater than about 200 μm) using Formula 1:

$$\eta = \left(\frac{\nu^3}{\epsilon}\right)^{\frac{1}{4}}$$

wherein ϵ is [U^3/L], L is the length of tubing connecting the bioreactor and RTF or ATF cell retention device, U is [flow rate in tubing/cross-sectional area of tubing], and ν is [cell culture viscosity / cell culture density]. Optionally, the conditions include a flow rate through the tubing selected from about 4 mL/min/fiber to about 16 mL/min/fiber for hollow fiber modules, about 360 L/m^2/hr to about 720 L/m^2/hr for flat sheet filter modules, or about 1 L/m/channel to about 4 L/m/channel for stacked plate modules; a cell culture viscosity selected from about 1 to about 6 centipoise (e.g., about 2 to about 6 centipoise); and/or a cell culture density is selected from about 1 g/L to about 1.5 g/L.

[0007] The disclosure further provides a formulation of etanercept comprising etanercept produced using the method described herein. In another aspect, the present disclosure relates to a formulation comprising etanercept produced using the method as disclosed herein. The formulation, in various examples, comprises: 50 mg/mL of etanercept, 1% sucrose (w/v), 100 mM sodium chloride, 25 mM sodium phosphate, 25 mM L-arginine, and water; 25 mg of etanercept, 40 mg mannitol, 10 mg sucrose, and 1.2 mg tromethamine in a lyophilized powder; or 50 mg/mL of etanercept, 1% sucrose (w/v), 120 mM sodium chloride, 25 mL L-arginine, and water. The disclosure also provides a method of treating a patient in need thereof, the method comprising administering to the patient an effective dose of etanercept produced using the method of disclosure. In another aspect, the present disclosure relates to etanercept produced using the method as disclosed herein for use in therapy. In various examples, the patient is suffering from rheumatoid arthritis, polyarticular juvenile idiopathic arthritis, psoriatic arthritis, ankylosing spondylitis, or plaque psoriasis. In another aspect, the present disclosure relates to the use of etanercept produced using the method as disclosed herein in the manufacture of a medicament for treating rheumatoid arthritis, polyarticular juvenile idiopathic arthritis, psoriatic arthritis, ankylosing spondylitis, or plaque psoriasis. Use of etanercept produced as described herein to treat a patient in need thereof, and use of etanercept produced as described herein in the preparation of a medicament to treat a patient in need thereof, are contemplated. A kit comprising the formulation described herein and instructions for use of the formulation is provided. In another aspect, the present disclosure relates to a kit comprising the formulation as disclosed herein and instructions for use of said formulation.

[0008] It should be understood that, while various examples in the specification are presented using "comprising" language, under various circumstances, a related example may also be described using "consisting of" or "consisting essentially of" language. The disclosure contemplates examples described as "comprising" a feature to include examples which "consist of" or "consist essentially of" the feature. The term "a" or "an" refers to one or more; the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein. The term "or" should be understood to encompass items in the alternative or together, unless context unambiguously requires otherwise. The term "and/or" should be understood to encompass each item in a list (individually), any combination of items a list, and all items in a list together.

[0009] It should also be understood that when describing a range of values, the disclosure contemplates individual values found within the range. For example, "cell aggregate size of between about 20 μm and about 200 μm," could be, but is not limited to, 40 μm, 60 μm, 100 μm, etc., and any value in between such values. In any of the ranges described herein, the endpoints of the range are included in the range. However, the description also contemplates the same ranges in which the lower and/or the higher endpoint is excluded.

[0010] Additional features and variations of the disclosure will be apparent to those skilled in the art from the entirety of this application, including the figures and detailed description, and all such features are intended as examples of the disclosure. Likewise, features of the disclosure described herein can be re-combined into additional examples that also are intended as examples of the disclosure, irrespective of whether the combination of features is specified as an example or example of the disclosure. The entire document is intended to be referred as a unified disclosure, and it should be understood that all combinations of features described herein (even if described in separate sections) are contemplated, even if the

combination of features is not found together in the same sentence, or paragraph, or section of this document. Also, only such limitations which are described herein as critical to the disclosure should be viewed as such; variations of the disclosure lacking limitations which have not been described herein as critical are intended as examples of the disclosure.

BRIEF DESCRIPTION OF THE FIGURES

[0011] Figure 1 provides an amino acid sequence of etanercept. It is to be understood that minor modifications and deletions of this sequence (up to 10%) may be possible and can be used within the scope of the disclosure. The method and techniques of the present disclosure can be used to produce biosimilar etanercept that differs from ENBREL® in, for example, its sequence, glycosylation pattern, or other post-translational modification.

[0012] Figure 2 is a graph illustrating data described in the Example. Final titer of etanercept (mg/L; y-axis) is provided for N-0 cultures inoculated from N-1 cultures performed under condition #1 (AML SSM 2019), condition #2 (ATF A2B=0.125), condition #3 (ATF A2B=0.25), and condition #4 (ATF A2B=0.375).

[0013] Figures 3A and 3B are schematics of a bioreactor system comprising a bioreactor operably coupled to a cell retention device. The cell retention device in the illustrative embodiment of Figure 3A is an alternating tangential flow filtration (ATF) system comprising at least one filter module and a pump. Figure 3B is an illustration of a bioreactor operably connected to a recirculating tangential flow filtration (RTF) system comprising at least one filter module and a pump. Media is introduced into the bioreactor while the cell retention device pump flows cell culture through the filter module. Cell-free harvest, which typically comprises spent media, culture waste, recombinant protein, etc., is removed. Tubing connects the media source to the bioreactor, the bioreactor to the cell retention device, and cell retention device to harvest collection means (represented by arrows in schematic). The schematics of Figures 3A and 3B are provided merely to illustrate representative aspects of the disclosure; the system and method of the disclosure are not dependent on a particular arrangement of components of an N-1 bioreactor system.

DETAILED DESCRIPTION

[0014] Despite the many years of research and development devoted to therapeutic products, efficient production of recombinant proteins on an industrial scale remains a challenge. Improvements to manufacturing that increase productivity, improve consistency of product, reduce cost of starting materials or processing time, or reduce equipment costs would have substantial economic benefits. Each step of the manufacturing process has the potential to affect the final product delivered to the patient, however, and the ultimate effect of adjusting parameters of a production method can be unpredictable, particularly when scaling up to industrial-scale processes. Indeed, the system and method of producing a recombinant protein affects not only the amount of protein produced by cell culture, but also the protein composition itself with respect to, e.g., glycosylation patterns, in potentially unpredictable ways. Further, the conditions appropriate for production of one recombinant protein are not necessarily appropriate for efficient, consistent manufacture of a different recombinant protein. The disclosure provides a system and method for producing etanercept in Chinese hamster ovary (CHO) cells that maximizes recombinant protein production while producing a consistent therapeutic product, overcoming technical hurdles associated with industrial scale production of therapeutic proteins.

[0015] Etanercept (Enbrel®, Immunex Corporation) is a dimeric fusion polypeptide consisting of the extracellular ligand-binding portion of the human 75 kilodalton (p75) tumor necrosis factor receptor (TNFR) linked to the Fc portion of human IgG1 (see, e.g., U.S. Pat. No. 9,518,111). The Fc component of etanercept contains the constant heavy 2 (CH2) domain, the constant heavy 3 (CH3) domain and hinge region, but not the constant heavy 1 (CH1) domain of human IgG1. It