INTEGRATED AND CONTINUOUS RECOMBINANT PROTEIN MANUFACTURING

FIELD OF THE SUBJECT TECHNOLOGY

The subject technology relates to methods of protein production in cultured animal cells, preferably mammalian cells, using a continuous cell-culture system and method.

BACKGROUND OF THE SUBJECT TECHNOLOGY

The current continuous cell-culture systems and methods (including perfusion or fed-batch bioreactors) suffer from many disadvantages, including a lack of scalability and low production yield. Therefore, there is a need for an alternative continuous cell-culture system or method that overcomes these limitations.

SUMMARY OF THE SUBJECT TECHNOLOGY

The goal of this subject technology is to overcome the current limitations of continuous cell-culture systems and methods.

One or more aspects of the present invention are described as numbered clauses (e.g., 1, 2, 3, etc), below for convenience. These clauses are provided as examples and not as limitations of the subject technology. Any dependent clause below may be included (by any combination), and placed into a respective independent clause, such as clause 1 from which clause 2 depends. The other clauses can be presented in a similar manner.

1. An integrated system for manufacturing a recombinant protein product, said system including a single-use bioreactor (SUB), a tangential flow filtration system, a column chromatography skid, a tankless hold, a virus inactivation plug flow reactor, and pumps.

2. The integrated system of clause 1, wherein system is automated, continuous and/or single-use.

3. The integrated system of clause 1, wherein each of the SUB, the tangential flow filtration system, the column chromatography skid, the tankless hold, the virus inactivation plug flow reactor and the pumps is single-use.

4. The integrated system of clause 1, wherein the SUB includes a perfusion bioreactor with a volume of about 10L to about 2250L.

5. The integrated system of clause 1, wherein the SUB includes a fed-batch bioreactor with a volume of about 2L to about 4500L.

6. The integrated system of clause 5, wherein the SUB is fluidically connected, without a tank and by tubing, to the tangential flow filtration system generating permeate.

7. The integrated system of clause 6, wherein the permeate is transferred by an about 1/4 inch to about 3/8 inch (or about 6 mm to about 10 mm) single-use tubing directly to the column chromatography skid without a tank.

8. The integrated system of clause 7, wherein the permeate is cooled to room temperature using a heat exchanger.

9. The integrated system of clause 1, wherein the column chromatography skid includes a dual-column capture chromatography, each generating effluent.

10. The integrated system of clause 9, wherein the first or capture column is a Protein A column.

11. The integrated system of clause 9, wherein the second column is an anion exchange column.

12. The integrated system of clause 9, wherein the column chromatography skid is fluidically connected, without a tank and by tubing, to the tankless hold.

13. The integrated system of clause 9, wherein the effluent is transferred by an about 1/4 inch to about 3/8 inch (or about 6 mm to about 10 mm) single-use tubing to the tankless hold.

14. The integrated system of clause 1, wherein the tankless hold is fluidically connected to the virus inactivation plug flow reactor (e.g., in serpentine or knot design forms as described in co-pending patent applications No. 62/742,530 and/or 62/742,534 filed

on October 8, 2018, the entire content of each of which is hereby incorporated herein by reference).

15. The integrated system of clause 1, wherein the pumps are fluidically connected to the SUB, tangential flow filtration system, column chromatography skid, tankless hold, and/or the virus inactivation plug flow reactor.

16. The integrated system of clause 1, wherein the pumps comprise a first set of pumps and a second set of pumps.

17. The integrated system of clause 16, wherein the first set of pumps operate with a range of about 60 mL/min to about 5 L/min or about 60 mL/min to about 600 mL/min.

18. The integrated system of clause 16, wherein the second set of pumps operate with a range of about 125 mL/min to about 2.5L/min.

19. The integrated system of clause 1, wherein the system is capable of processing between 10g and 5,000g of recombinant Protein per day.

20. An automated integrated single-use continuous cell culture system including:

    a. a single-use bioreactor (SUB) between 10L and 2250L,
    b. a single-use tangential flow filtration system generating a permeate,
    c. a single-use dual-column capture column chromatography skid generating an effluent;
    d. a tankless hold and
    e. a single-use virus inactivation plug flow reactor; and
    f. a first set of pumps operating with a range of about 60 mL/min to about 5 L/min, or about 60 mL/min to about 600 mL/min and a second set of pumps operating with a range of about 125 mL/min to about 2.5 L/min;

    wherein the single-use SUB is fluidically attached, without a tank and by tubing, to the single-use tangential flow filtration system; wherein the permeate is transferred, without a tank, by an about 1/4 inch to about 3/8 inch (or about 6 mm to about 10

mm) single-use tubing to the single-use dual-column capture column chromatography skid; wherein the effluent is transferred, without a tank, by an about 1/4 inch to about 3/8 inch (or about 6 mm to about 10 mm) single-use tubing to the tankless hold; wherein the tankless hold is fluidically connected to the single-use virus inactivation plug flow reactor; wherein the first and second sets of pumps are fluidically connected to one or more of components a-e.

21. The automated integrated single-use continuous cell culture system of clause 20, wherein the system is capable of processing between 10g and 5,000g of recombinant protein per day.

22. The automated integrated single-use continuous cell culture system of clause 20, wherein the SUB is a perfusion bioreactor or a fed-batch bioreactor.

23. A lab-scale automated integrated single-use continuous cell culture system, including:

    a. a single-use bioreactor (SUB) between 10L and 2250L,
    b. a single-use tangential flow filtration system generating a permeate,
    c. a single-use dual-column capture column chromatography skid generating an effluent;
    d. a tankless hold and
    e. a single-use virus inactivation plug flow reactor; and
    f. a first set of pumps operating with a range of about 0.060mL/min to about 5mL/min, or about 0.060mL/min to about 0.6600mL/min and a second set of pumps operating with a range of about 0.125mL/min to about 2.5mL/min.

24. The lab-scale automated integrated single-use continuous cell culture system of clause 23, wherein the single-use SUB is fluidically attached, without a tank and by tubing, to the single-use tangential flow filtration system; wherein the permeate is transferred, without a tank, by about 0.012 into about 0.036-inch (or about 0.3 to about 1 mm) single-use tubing to the single-use dual-column capture column chromatography skid.

25. The lab-scale automated integrated single-use continuous cell culture system of clause 24, wherein the effluent is transferred, without a tank, by single-use 0.012 into 0.036 inch (or about 0.3 to about 1 mm) tubing to the tankless hold; wherein the tankless hold is fluidically connected to the single-use virus inactivation plug flow reactor.

26. The lab-scale automated integrated single-use continuous cell culture system of clause 25, wherein the first and second sets of pumps are fluidically connected to one or more of components a-e.

27. The lab-scale automated integrated single-use continuous cell culture system of clause 23, wherein the SUB is a perfusion bioreactor or fed-batch bioreactor.

28. A lab-scale automated integrated single-use continuous cell culture system including:

    a. a single-use bioreactor (SUB) between 10L and 2250L,
    b. a single-use tangential flow filtration system generating a permeate,
    c. a single-use dual-column capture column chromatography skid generating an effluent;
    d. a tankless hold and
    e. a single-use virus inactivation plug flow reactor; and
    f. a first set of pumps operating with a range of about 0.060mL/min to about 5mL/min, or about 0.060mL/min to about 0.6600mL/min and a second set of pumps operating with a range of about 0.125mL/min to about 2.5mL/min;

    wherein the single-use SUB is fluidically attached, without a tank and by tubing, to the single-use tangential flow filtration system; wherein the permeate is transferred, without a tank, by about 0.012 into about 0.036 inch (or about 0.3 to about 1 mm) single-use tubing to the single-use dual-column capture column chromatography skid; wherein the effluent is transferred, without a tank, by single-use 0.012 into 0.036 inch (or about 0.3 to about 1 mm) tubing to the tankless hold; wherein the tankless hold is fluidically connected to the single-use virus inactivation plug flow

reactor; wherein the first and second sets of pumps are fluidically connected to one or more of components a-e.

29. The lab-scale automated integrated single-use continuous cell culture system of clause 28, wherein the SUB is a perfusion bioreactor or a fed-batch bioreactor.

30. The integrated system according to any of the preceding clauses, wherein the system is mounted on wheels.

31. The integrated system according to any of the preceding clauses, wherein the systems makes about 5 to about 60 kg of recombinant Protein in about 10 to 20 days.

Additional features and advantages of the subject technology will be outlined in the description below will be apparent from the description or learned by practice of the subject technology. The advantages of the subject technology are presently described and embodiments may be envisioned by a person of skill in the art of the technology as particularly pointed out in the written description and claims hereof and as described in the appended drawings.

The foregoing general description and the following detailed description are exemplary and explanatory and are intended to provide further explanation of the subject technology as claimed.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features, aspects, and advantages of the claimed methods, apparatuses, and systems are better understood when the following detailed description is read with reference to the accompanying drawings:

FIG. 1 shows viable cell density (solid lines, primary y-axis) and viability (dashed lines, secondary y-axis) for three pilot-scale iSkid runs: 1 (Δ), 2 (□), and 3 (○). All runs performed at 100L running a mammalian cell line expressing a recombinant monoclonal antibody over 14 days. Dotted lines represent +/- 3 standard deviations.

FIG. 2 illustrates a representative elution chromatogram for the Protein A step.

FIG. 3 shows a representative chromatogram for the Anion-Exchange step.

FIG. 4 shows a representative pH trace of material entering the plug flow reactor.

Fig. 5 shows the process flow.

Fig. 6 shows a schematic of the design, including the plug flow reactor (labeled as an incubation chamber) and the tankless hold (labeled as Elution Stream Chamber). The Single-Pass Tangential Flow Filtration system is not discussed in the application.

Fig 7. Shows the host cell protein values for BI-5 runs 1 and 2 as a function of the ProA cycle number. The results are shown after three different unit operations: perfusion permeate, post ProA chromatography, and Post AEX chromatography. CHOP stands for Chinese hamster ovarian protein or host cell protein.

Fig 8. Shows the low molecular weight (LMW) and high molecular weight (HMW) values for BI% as a function of cycle number. The samples were taken from the single-use mixer. CR stands for consistency run.

Fig.9. Shows an example of the yield measurement for the Anion Exchange step demonstrated for a full run.

DETAILED DESCRIPTION OF THE SUBJECT TECHNOLOGY

Without wishing to be bound by any theory, the present invention is in part based on the surprising finding that the automated recombinant manufacturing system of the subject technology with a specific set of single-use pump sizes, tubing sizes, and column sizes, and the use of a single-use reactor ("Tankless Hold"), a single-use buffer concentrates dilution system and no surge tanks between the unit operations will have a 500-fold dynamic range. The current perfusion systems have only a 10-20 dynamic range.

In one aspect, the subject technology relates to an automated integrated single-use system controlling a single-use perfusion or fed-batch bioreactor (SUB) between 10L and 2250L attached by tubing to a single-use tangential flow filtration system, the permeate of which is directly attached by 1/4 inch to 3/8 inch single-use tubing to a single-use dual-column capture chromatography skid, the product effluent of which is attached without a tank by single-use 1/4 inch to 3/8 inch tubing to a tankless hold and a single-use virus inactivation plug flow reactor, the

product effluent of which is attached by 1/4 inch to 3/8 inch (or about 6 mm to about 10 mm in diameter) tubing to a flow-through chromatographic step. The system uses a single-use buffer supply delivery system with a set of single-use pumps with a range of 60mL/min to 600mL/min and a set of pumps with a range of 125mL/min to 2.5L/min that is capable of processing between 10g and 5,000g of recombinant protein per day. As used herein, the term "tank" refers to a vessel that is capable of holding and mixing liquid and has an air-liquid interface during use.

In one embodiment, the SUB equipment can be changed to another SUB of different volume between 10 and 2250L without an other equipment change to the rest of the skid. The SUB equipment is defined as the durable components, which include the SUB shell, the mass flow controllers for O2, CO2, and air, the recirculation pump, and the permeate pump. The single-use assemblies, such as the chromatography columns, any product contact material, the flow meter, will change size depending upon the mass and volume of material to be processed. For instance, a 100L SUB could typically require dual 1L capture columns and between about 1.1 and about 3L tankless hold, while a 2000L SUB would require dual 13L capture columns, and between about 14L and about 39L tankless hold.

In another embodiment, the tankless hold has a volume of 1.1 to 3 times the affinity column volume.

In another embodiment, the buffer supply system and associated valves and pumps that allow the dilution of buffer solutions from a buffer concentrate of five times the process buffer concentration, and capable of processing of small amounts (50g) to large amounts (5kg) per day without loss of efficiency or material.

In another embodiment, a single-use heat exchanger is placed in the permeate line between the TFF and the dual capture columns. The heat exchanger is sized such that it is capable of reducing the temperature of the permeate from approximately 37 °C to 20-25 °C. The size of the heat exchanger depends upon the flow rate and is obvious to those skilled in the art.

In another embodiment, the solution supply to capture step includes two pumps, one of which is attached to one water/diluent line, and the other pump to 3 to 8 buffer concentrates, one of which contains sanitization fluid.

In another embodiment, the solution supply to polishing step includes two pumps, one of which is attached to one water/diluent line, and the other pump is attached to 3 to 9 buffer concentrates, one of which is attached to one water/diluent line, and the other to 3 to 8 buffer concentrates, one of which contains sanitization fluid.

In another embodiment, the solution supply to virus inactivation step contains two buffer lines and at least one line for sanitization.

In another embodiment, the solution supply to SPTFF has one pump, and one to two buffer concentrates, and one line for sanitization.

In another embodiment, the pump characteristic includes single-use pumps capable of flow rates between 100mL/min and 10 L/min. A single pump is typically able to operate accurately over a range of about 20x, from 5% of maximum pump capacity to 95% of maximum pump capacity. Thus, a pump with a capacity of 0 LPM to 10 LPM can operate accurately between about 0.5 LPM and 9.5 LPM. A pump can operate even more accurately between 10% and 90% of the maximum pump capacity, a range of about 10x. The use of buffer concentrates means that the most accurate pumps must be the buffer concentrate pumps, which should remain within a 10-90% of maximum pump capacity. A 5% difference in a 5x buffer concentrate flow rate results in a buffer that is 20% different than intended, which is typically too large for most bioprocesses.

In another embodiment, each pair of pumps has the buffer concentrate pump with a range of 60 mL/min to 600 mL/min (ideally for buffer concentrates) and another set of pumps of 125 mL/min to 2.5 L/min, primarily for diluent.

In another embodiment, at smaller scales, the buffers could be made at 1x. At smaller scales, a 1x solution is used, and only the buffer pumps are used. Those skilled in the art recognize these pump sizes as preferred. The pump for the buffer concentrate and the diluent can have the same capacity, with only a marginal impact on the total dynamic range of capacities.

Table 1 below shows the range of capabilities of a described based on the parameters listed in Table 2. The minimum practical daily mass is higher than the Minimum Daily Mass due to considerations of the column volume compared to the tubing. In this case, the column volume is

only about 6x that of the tubing volume (1m of tubing as an example). This extra column volume would cause significant dilution of the product in the iskid, and may lead to yield loss.

Table 1.

Minimum Bioreactor Size (approx)	Max Perfusion Bioreactor	Max Fed-Batch Bioreactor	Minimum daily mass	Max Daily Mass	Min Column Size	Max Column Volume
10mL	2L	5L	0.05 g	5g	0.25mL	13mL
10.0L	2250L	4500L	50 g	5 kg	250mL	13L

Table 1. Continues

Buff Pump Lower Limit	Buff Pump Upper Limit	Diluent Pump Lower Limit	Diluent Pump Upper Limit	Min Tubing size	Max Tubing Size
0.06 mL/min	0.63 mL/min	0.13 mL/min	2.50 mL/min	0.012 in (300um)	0.036 in (910um)
0.063 L/min	0.63 L/min	0.125 L/min	2.5 L/min	0.25 in	0.75 in

Table 2: Assumptions/Parameters

Minimum Capture step Capacity	20 g/L resin
Maximum Capture Step Capacity	50 g/L resin
Min cycles/day	2
Max cycles/day	8
Column Residence Time	4 Min
Max flowrate in 1/4 in tubing	3.13L/min
Maximum Buffer Concentrate	5x over process concentrate

Various other examples or embodiments relating to one or more aspects of the present invention are described as numbered clauses (1, 2, 3, etc.) below for convenience. These are provided as examples and do not limit the subject technology. As previously noted, any of the dependent clauses may be combined in any combination, and placed into a respective independent clause, e.g., clause 1. The other clauses can be presented in a similar manner.

1. An automated integrated single-use system controlling a single-use perfusion bioreactor (SUB) between 10L and 2250L attached by tubing to a single-use tangential flow filtration system, the permeate of which is attached without a tank by 1/4 inch to 3/8 inch single-use tubing to a single-use dual-column capture chromatography skid, the product effluent of which is attached without a tank by single-use 1/4 inch to 3/8 inch tubing to a tankless hold and a single-use virus inactivation plug flow reactor, with sets of single-use pumps with a range of 60mL/min to 5L/min, or preferably 60mL/min to 600mL/min and a set of pumps with a range of 125mL/min to 2.5L/min.

2. The integrated system of clause 1, and the product effluent of which is attached by 1/4 inch to 3/8 inch tubing to a chromatographic step.

3. The integrated system of clause1 or 2 and the system a single-use buffer supply delivery system with a set of single-use pumps with a range of 60mL/min to 600mL/min and a set of pumps with a range of 125mL/min to 2.5L/min that is capable of processing between 10g and 5,000g of recombinant protein per day.

4. The integrated system wherein the capture column is a Protein A column.

5. The integrated system where the second column is an anion exchange column.

6. An automated integrated single-use system controlling a single-use fed-batch bioreactor (SUB) between 2L and 4500L attached to a cell removal device, the effluent of which is attached by ¼ inch to 3/8 inch single-use tubing to a single-use dual-column capture chromatography skid, the product effluent of which is attached without a tank by single-use ¼ inch to 3/8 inch tubing to a tankless hold and a single-use virus inactivation plug flow reactor, the product effluent of which is attached by ¼ inch to 3/8 inch tubing to a chromatographic step. The system uses a single-use buffer supply delivery system with a set of single-use pumps with a range of 60mL/min to 600mL/min and a set of pumps with a range of 125mL/min to 2.5L/min that is capable of processing between 10g and 5,000g of recombinant Protein per day.

7. A lab-scale automated integrated single-use system controlling a single-use perfusion bioreactor (SUB) between 10mL and 2250mL attached by tubing to a single-use tangential flow filtration system, the permeate of which is attached without a tank by 0.012 into

0.036-inch single-use tubing to a single-use dual-column capture chromatography skid, the product effluent of which is attached without a tank by single-use 0.012 into 0.036-inch tubing to a tankless hold, with sets of single-use pumps with a range of 0.060mL/min to 5mL/min, or preferably 0.060mL/min to 0.6600mL/min and a set of pumps with a range of 0.125mL/min to 2.5mL/min.

8. The integrated lab-scale system of Clause7 with a single-use virus inactivation plug flow reactor

9. The integrated system of clause 7, and the product effluent of which is attached by 0.012 into 0.036-inch tubing to a chromatographic step.

10. The integrated system of Clause7 or 8 or 9 and the system a single-use buffer supply delivery system with a set of single-use pumps with a range of 0.06mL/min to 0.600mL/min and a set of pumps with a range of .125mL/min to 2.5mL/min that is capable of processing between 10mg and 5,000mg of recombinant Protein per day.

11. The integrated system of claims 1-6 mounted on wheels.

12. A method, whereby the integrated skid system from claims 1-7 makes 5, 10, 15, 20, 30, 40 60kg of antibody compounds in 10, 14, 20 days.

13. The use of the skid device/system above to make 5, 10, 15, 20, 30, 40 60kg of recombinant Protein in 10, 14, 20 days.

14. The use of 5x buffer concentrates in single-use vessels.

15. The use of 10x buffer concentrates in single-use vessels.

16. The use of portable bioreactors with various volumes that can be plugged into the system (for example, 100L, 500L, 1000L, or 2000L single-use bioreactors).

17. The use of sanitization solution (such as 0.5M NaOH for 15 minutes) two times per day in the capture step, the plug flow reactor, and the polishing step

Examples

Example 1

An automated single-use system

An automated single-use system controlled a 100L single-use perfusion bioreactor (SUB) attached by 3/4 inch tubing to a single-use tangential flow filtration system with a recirculation pump (Levitronics Pump and Spectrum 0.2um TFF filter), and a permeate pump (Quatroflo 150).

The permeate was attached by 1/4 inch single-use tubing to a single-use dual-column 1L MabSelect Sure LX Protein A (GE Healthcare) column on a chromatography skid or capture step. Each column operation consisted of standard phases for Protein A steps, which are known to those skilled in the art that include a loading phase, three wash phases, an elution phase, a strip phase, a sanitization phase, an equilibration phase, and (optionally) a hold phase. The buffers and volumes used are shown in Table 2. The solutions were diluted from buffer concentrates by water. Table 2 below lists protein A buffers, volumes, and operating parameters. The concentration of the buffers is shown after dilution. The concentration of the buffers before dilution was five-fold higher, except for the Wash 2 solution, which was two-fold higher.

Table 2.

Step Description	**Solution Description**	**CVs**	**Residence time (min)**	**Flow Rate (mL/min per 1L CV)**
Pre-Load Equilibration	50 mM Tris, 150 mM NaCl, pH 7.5	5	5	< 251
Load	Perfusion bioreactor permeate after passing through a 0.2um filter	varies	≥4	≤ 208 mL/min
Wash 1	50 mM Tris, 150 mM NaCl, pH 7.5	2	5	≤ 200
Wash 2	50 mM Tris, 0.5 M $CaCl_2$, pH 7.5	5	4	≤ 250
Wash 3	10 mM Tris, 10 mM NaCl, pH 7.5	3	4	≤ 250

Step Description	Solution Description	CVs	Residence time (min)	Flow Rate (mL/min per 1L CV)
Elution	25 mM Acetate, pH 3.50 ± 0.10	5 (Product elution is equivalent to 3 CVs - detailed in section 2.2.3)	5	≤ 200
Strip	250 mM Acetate, 250mM NaCl	5	4	≤ 250
Sanitization	0.5 M NaOH	4	5 (15 to 20 minute total contact time)	≤ 200
Equilibration	50 mM Tris, 150 mM NaCl, pH 7.5	3	5	≤ 200
Hold	50 mM Tris, 150 mM NaCl, pH 7.5	0	n/a	0

Each column of the dual column capture step is operated in parallel, with one loading, while the other one goes through the other phases.

The effluent of the elution phase was directed by means of valves and 1/4 inch tubing to a 3L tankless hold. The product effluent is held in the tankless hold until the plug flow reactor was ready to operate. The plug flow reactor was first primed with 50mM glycine pH 3.3 to 3.5. When the plug flow reactor is ready, the product was pumped from the tankless hold at 50mL/min into the plug flow reactor while simultaneously a stream of 2 M glycine pH 3.3 flowing at 7.5mL/min was mixed with the product. The resulting product stream had a pH between 3.3 and 3.5. Once the product stream was completely removed from the tankless hold and contained in the plug flow reactor, the tankless hold effluent was switched to drain by valves. The tankless hold was then flushed and sanitized with a solution of 0.5M NaOH for 30 minutes. Simultaneously, a series of valves were switched, another pump was turned on and maintained the flow in the plug flow reactor at 50mL/min by pumping 50mM glycine pH 3.3 into the plug flow reactor, thus effecting

the passage of the product through the plug flow reactor. When the UV signal on the effluent of the plug flow reactor rose above 100mAU, the effluent was diverted from going to a sanitary drain and was directed to an anion exchange column through a ¼ inch tube while neutralization solution consisting of 315mM Tris base was added to the PFR product effluent at a rate of 58mL/min. The product stream passed through a static mixer before passing onto a 500mL anion exchange column.

After the product stream had completely exited the plug flow reactor, the effluent from the plug flow reactor was directed to waste. The system was flushed with a sanitizing solution of 0.5M NaOH for at least 30 minutes before the next product cycle.

The anion exchange column was operated in a weak partitioning chromatographic mode familiar to those skilled in the art, such that the product mainly passes through the column during the load and subsequent wash step. The conditions of the anion exchange column are shown in Table 3. Table 3 lists anion exchange buffers, volumes, and operating parameters. The concentration of the buffers is shown after dilution. The concentration of the buffers before dilution was five-fold higher, except for the sanitization solution, which was two-fold higher.

Table 3.

Step Description	**Solution Description**	**AEXCVs[1]**	**Residence time (minutes)**	**Flow rate (mL/min)**
Equilibration	50 mM Tris, pH 8.1	5	3.2	126
Chase	50 mM Tris, pH 8.1	3	3.2	126
Sanitization (total sanitization time 30 min)	0.5 M NaOH, 2 M NaCl	5	8	67
Equilibration	50 mM Tris, pH 8.1	5	3.2	126

The product effluent from the anion exchange was directed to a single pass tangential flow (SPTFF) step through 1/4 inch tubing. The SPTFF step concentrates the product using an ultrafiltration step familiar to those skilled in the art. The permeate flow rate from the SPTFF was about 58mL/min, and the concentrated product was directed by valves to mix with 10% by volume, a 1M MES pH 6.0 solution. The mixture was then directed by valves through ¼ inch tubing into

a 100L single-use mixer or single-use tank. After the product stream passed through the SPTFF, the effluent of the SPTFF was diverted to waste, and the system flushed with a sanitizing solution of 0.5M NaOH for over 30 minutes.

The system operated for 14 days and achieved a cell density of about 150 million cells per mL, as shown in Figure 1. Representative chromatograms are shown for the Protein A step, Figure 2, and the Anion Exchange step in Figure 3. A representative pH trace of material entering and exiting the plug flow reactor is shown in Figure 4.

Example 2

Process Summary

The diagram shown in Figure 5 describes the process for the production of >1000 g of formulated bulk drug substance utilizing the 100 L single-use perfusion bioreactor system. The current state of the technology requires that the seed train expansion process to be according to specific cell line requirements and will rely on existing fed-batch procedures.

Example 3

Purification Process Summary

The process described here is for the downstream processing of a mAb. The downstream process is designed for a continuous harvest stream productivity that ranges from 0.4 – 3.3 g/L/d or 0.8-6.6 g/L/d at a maximum flow rate of 3 bioreactor volumes per day. Downstream processing occurs from 8-13 days. See Figure 6.

While in the current example a mixture of sodium and potassium carbonate added continuously were used to provide the upward pressure on pH that the consumption of lactic acid from the perfusion medium would have supplied if sodium-L-lactate were in the perfusion medium, presumably any appropriate non-toxic basic substance added in a continuous or semi-continuous manner to the culture could provide the same effect. Examples of such bases could include sodium or potassium hydroxide, among many others.

While the subject technology has been described in detail and with reference to specific embodiments thereof, it will be apparent to one of ordinary skill in the art that various changes and modifications can be made to the claimed invention without departing from the spirit and scope thereof. Thus, for example, those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of the subject technology and are covered by the claims.

Example 4

Summary of Product Quality across different mAbs

Extensive development and testing of the integrated system of the present invention for the manufacturing of a recombinant protein product, which we call iSKID™, has demonstrated that the technology is robust and reliable. The operation of pilot-scale prototype systems with 100-liter bioreactors have demonstrated consistent operational performance. The system has been tested using several cell lines and multiple production runs.

Our findings have shown that there are no differences in product quality for drug substances produced using the iSKID™ as compared to those produced using a conventional fed-batch process. As a demonstration of the capability of the iSKID™ to provide high-quality material, the data obtained from the prototype systems represents cell lines producing four monoclonal antibodies: STL-B, AND-T, BI-5, and BI-10. All molecules were manufactured using the iSKID™ systems without any specific product development. All materials or drug substances were generated in a non-GMP environment for information gathering only. A summary of the product quality data in drug bulk substance is shown in Table 4. The data shows that the product quality across products is appropriate for clinical use. Run to run (or batch to batch) consistency was demonstrated for the products that ran more than once in the iSKID™ under consistent conditions.

Table 4.

Molecule name		STL-B	AND-T	BI-5	BI-10
Number of Runs		3	3	2**	1
Analytical Procedure	**Quality Targets**	**Batch Results**	**Batch Results**	**Batch Results**	**Batch Results**
Characteristics					
iCE	Report results				
	% Main Peak (≥ 50.0%)	61±0.7%	63±5.4%	45±1.4%	67.6%
	% Acidic Peaks (≤ 50.0%)	16±0.0%	12±2.6%	10±1.2%	20.4%
	% Basic Peaks (≤ 45.0%)	23±0.6%	25±4.7%	45±1.8%*	11.9%
Purity					
Size Exclusion HPLC	≥ 95.0 % monomer	98.7±0.6%	96.5±0.2%	97.9±0.5%	99.0%
CGE (reducing)	≥ 90.0 % IgG as heavy + light chain	99.2±0.4%	96.3±0.6%	98.3±0.0%	99.2%
Product-Related Impurities					
Size Exclusion HPLC	≤ 5.0 % HMMS	0.9±0.1%	3.4±0.3%	2.0±0.5%	1.0%
CGE (reducing)	≤ 6.0 % (fragments)	0.4±0.2%	1.6±0.6%	2.0±0.0%	0.8%
Process-Related Impurities					
HCP ELISA	≤ 100 ng/mg	<0.3	≤ 0.3	<1	<0.3

Abbreviations: iCE = Imaged Capillary Isoelectric Focusing; HMMS = High Molecular Mass Species; CGE = Capillary Gel Electrophoresis; HCP = Host Cell Protein; ELISA = Enzyme Linked Immunosorbent Assay; NMT = Not More Than
*Elevated species were fully characterized and assigned as non CQAs
**Pending results from consistency run 3 for BI-5
95% confidence intervals were used to determine batch ranges

The product quality profiles at different stages of the periodic stage for selected attributes for the BI-5 molecule also illustrate consistency from run to run (Figure 7, Figure 8).

Example 5

Capabilities of the iSKID™ to measure yield

Because one central automation system is used in the iSKID™, it is possible to generate data in real-time, which in turn allows for yield calculations in real-time. This calculation is typically needed for formal submissions to regulatory agencies. Typically, this calculation is difficult to perform in real-time. By using the iSKID™, we have demonstrated the readily available yield per unit operation in the periodic stage. An example of the yield measurement for the Anion Exchange step is demonstrated for a full run (Figure 9).

INDUSTRIAL APPLICABILITY

The device and methods disclosed herein are useful for perfusion biomaufacturing, and thus for improving industrial methods for manufacturing recombinant, therapeutic proteins.

CLAIMS

What is claimed is:

1. An integrated system for manufacturing a recombinant protein product, said system comprising a single-use bioreactor (SUB), a tangential flow filtration system, a column chromatography skid, a tankless hold, a virus inactivation plug flow reactor, and pumps.

2. The integrated system of claim 1, wherein system is automated, continuous and/or single-use.

3. The integrated system of claim 1, wherein each the SUB, the tangential flow filtration system, the column chromatography skid, the tankless hold, the virus inactivation plug flow reactor and the pumps is single-use.

4. The integrated system of claim 1, wherein the SUB comprises a perfusion bioreactor with a volume of about 2L to about 2250L.

5. The integrated system of claim 1, wherein the SUB comprises a fed-batch bioreactor with a volume of about 2L to about 4500L.

6. The integrated system of claim 5, wherein the SUB is fluidically connected, without a tank and by tubing, to the tangential flow filtration system generating permeate.

7. The integrated system of claim 6, wherein the permeate is transferred by an about 1/4 inch to about 3/8 inch single-use tubing directly to the column chromatography skid without a tank.

8. The integrated system of claim 7, wherein the permeate is cooled to room temperature using a heat exchanger.

9. The integrated system of claim 1, wherein the column chromatography skid comprises a dual-column capture chromatography, each generating effluent.

10. The integrated system of claim 9, wherein the first or capture column is a Protein A column.

11. The integrated system of claim 9, wherein the second column is an anion exchange column.

12. The integrated system of claim 9, wherein the column chromatography skid is fluidically connected, without a tank and by tubing, to the tankless hold.

13. The integrated system of claim 9, wherein the effluent is transferred by 1/4 inch to 3/8 inch single-use tubing to the tankless hold.

14. The integrated system of claim 1, wherein the tankless hold is fluidically connected to the virus inactivation plug flow reactor.

15. The integrated system of claim 1, wherein the pumps are fluidically connected to the SUB, tangential flow filtration system, column chromatography skid, tankless hold, and/or the virus inactivation plug flow reactor.

16. The integrated system of claim 1, wherein the pumps comprise a first set of pumps and a second set of pumps.

17. The integrated system of claim 16, wherein the first set of pumps operate with a range of about 60 mL/min to about 5 L/min or about 60 mL/min to about 600 mL/min.

18. The integrated system of claim 16, wherein the second set of pumps operate with a range of about 125 mL/min to about 2.5L/min.

19. The integrated system of claim 1, wherein the system is capable of processing between 10g and 5,000g of recombinant Protein per day.

20. An automated integrated single-use continuous cell culture system comprising:

    a. a single-use bioreactor (SUB) between 10L and 2250L,
    b. a single-use tangential flow filtration system generating a permeate,

c. a single-use dual-column capture column chromatography skid generating an effluent;
d. a tankless hold and
e. a single-use virus inactivation plug flow reactor; and
f. a first set of pumps operating with a range of about 60 mL/min to about 5 L/min, or about 60 mL/min to about 600 mL/min and a second set of pumps operating with a range of about 125 mL/min to about 2.5 L/min;

wherein the single-use SUB is fluidically attached, without a tank and by tubing, to the single-use tangential flow filtration system; wherein the permeate is transferred, without a tank, by 1/4 inch to 3/8 inch single-use tubing to the single-use dual-column capture column chromatography skid; wherein the effluent is transferred, without a tank, by single-use 1/4 inch to 3/8 inch tubing to the tankless hold; wherein the tankless hold is fluidically connected to the single-use virus inactivation plug flow reactor; wherein the first and second sets of pumps are fluidically connected to one or more of components a-e.

21. The automated integrated single-use continuous cell culture system of claim 20, wherein the system is capable of processing between 10g and 5,000g of recombinant protein per day.

22. The automated integrated single-use continuous cell culture system of clause 20, wherein the SUB is a perfusion bioreactor or a fed-batch bioreactor.

23. A lab-scale automated integrated single-use continuous cell culture system comprising:

a. a single-use bioreactor (SUB) between 10L and 2250L,
b. a single-use tangential flow filtration system generating a permeate,
c. a single-use dual-column capture column chromatography skid generating an effluent;
d. a tankless hold and
e. a single-use virus inactivation plug flow reactor; and

f. a first set of pumps operating with a range of about 0.060mL/min to about 5mL/min, or about 0.060mL/min to about 0.6600mL/min and a second set of pumps operating with a range of about 0.125mL/min to about 2.5mL/min.

24. The lab-scale automated integrated single-use continuous cell culture system of claim 23, wherein the single-use SUB is fluidically attached, without a tank and by tubing, to the single-use tangential flow filtration system; wherein the permeate is transferred, without a tank, by about 0.012 into about 0.036-inch single-use tubing to the single-use dual-column capture column chromatography skid.

25. The lab-scale automated integrated single-use continuous cell culture system of claim 24, wherein the effluent is transferred, without a tank, by single-use 0.012 into 0.036-inch tubing to the tankless hold; wherein the tankless hold is fluidically connected to the single-use virus inactivation plug flow reactor.

26. The lab-scale automated integrated single-use continuous cell culture system of claim 25, wherein the first and second sets of pumps are fluidically connected to one or more of components a-e.

27. The lab-scale automated integrated single-use continuous cell culture system of clause 23, wherein the SUB is a perfusion bioreactor or fed-batch bioreactor.

28. A lab-scale automated integrated single-use continuous cell culture system comprising:

a. a single-use bioreactor (SUB) between 2L and 2250L,
b. a single-use tangential flow filtration system generating a permeate,
c. a single-use dual-column capture column chromatography skid generating an effluent;
d. a tankless hold and
e. a single-use virus inactivation plug flow reactor; and
f. a first set of pumps operating with a range of about 0.060mL/min to about 5mL/min, or about 0.060mL/min to about 0.6600mL/min and a second set of pumps operating with a range of about 0.125mL/min to about 2.5mL/min;

wherein the single-use SUB is fluidically attached, without a tank and by tubing, to the single-use tangential flow filtration system; wherein the permeate is transferred, without a tank, by about 0.012 into about 0.036-inch single-use tubing to the single-use dual-column capture column chromatography skid; wherein the effluent is transferred, without a tank, by single-use 0.012 into 0.036-inch tubing to the tankless hold; wherein the tankless hold is fluidically connected to the single-use virus inactivation plug flow reactor; wherein the first and second sets of pumps are fluidically connected to one or more of components a-e.

29. The integrated system according to any of the preceding claims, wherein the system is mounted on wheels.

30. The integrated system according to any of the preceding claims, wherein the systems makes about 5 to about 60 kg of recombinant Protein in about 10 to 20 days.

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