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

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

One or more examples of the present disclosure 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.

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.

In one aspect, the present disclosure provides 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.

In another aspect, the present disclosure provides 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.

In another aspect, the present disclosure provides 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.

In another aspect, the present disclosure provides 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.

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 examples 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.

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 2L to 2250L.

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

6. The integrated system of claim 1, wherein the SUB comprises a fed-batch bioreactor with a volume of 2L to 4500L,
   wherein the SUB is fluidically connected, without a tank and by tubing, to the tangential flow filtration system generating permeate,
   wherein the permeate is transferred by an 1/4 inch to 3/8 inch single-use tubing directly to the column chromatography skid without a tank, and/or
   wherein the permeate is cooled to room temperature using a heat exchanger.

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

8. The integrated system of claim 1, wherein the column chromatography skid comprises a dual-column capture chromatography, each generating effluent,
wherein the first or capture column is a Protein A column,
wherein the second column is an anion exchange column,
wherein the column chromatography skid is fluidically connected, without a tank and by tubing, to the tankless hold, and/or
wherein the effluent is transferred by 1/4 inch to 3/8 inch single-use tubing to the tankless hold.

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

10. 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.

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

12. The integrated system of claim 1, wherein the pumps comprise a first set of pumps and a second set of pumps,
wherein the first set of pumps operate with a range of 60 mL/min to 5 L/min or 60 mL/min to 600 mL/min, and/or
wherein the second set of pumps operate with a range of 125 mL/min to 2.5L/min.

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

14. 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 60 mL/min to 5 L/min, or 60 mL/min to 600 mL/min and a second set of pumps operating with a range of 125 mL/min to 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.

15. The automated integrated single-use continuous cell culture system of claim 14, wherein the system is capable of processing between 10g and 5,000g of recombinant protein per day, and/or

wherein the SUB is a perfusion bioreactor or a fed-batch bioreactor.

16. 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 0.060mL/min to 5mL/min, or 0.060mL/min to 0.6600mL/min and a second set of pumps operating with a range of 0.125mL/min to 2.5mL/min.

17. The lab-scale automated integrated single-use continuous cell culture system of claim 16, 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 0.012 into 0.036-inch single-use tubing to the single-use dual-column capture column chromatography skid.

18. The lab-scale automated integrated single-use continuous cell culture system of claim 16, 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 0.012 into 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, and/or
wherein the first and second sets of pumps are fluidically connected to one or more of components a-e.

19. The lab-scale automated integrated single-use continuous cell culture system of claim 16, wherein the SUB is a perfusion bioreactor or fed-batch bioreactor.

20. 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 0.060mL/min to 5mL/min, or 0.060mL/min to 0.6600mL/min and a second set of pumps operating with a range of 0.125mL/min to 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 0.012 into 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.

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

22. The integrated system according to any of the preceding claims, wherein the system makes 5 to 60 kg of recombinant protein in 10 to 20 days.