ACOUSTIC PERFUSION DEVICES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 62/563,068, filed on September 26, 2017, and to U.S. Provisional Patent Application Serial No. 62/482,681, filed on April 26, 2017. This application is also a continuation-in-part of U.S. Patent Application Serial No. 15/696,176, filed September 5, 2017. These applications are incorporated herein by reference in their entireties.

BACKGROUND

[0002] One of the key components that is utilized in the manufacturing processes of new biologically based pharmaceuticals is the bioreactor and the ancillary processes associated therewith. An area of growth in the bioreactor field has been with the perfusion process. The perfusion process is distinguished from the fed-batch process by its lower capital cost and continuous (rather than batch) operation.

[0003] A perfusion bioreactor processes a continuous supply of fresh media that is fed into the bioreactor while growth-inhibiting byproducts are constantly removed. The nonproductive downtime can be reduced or eliminated with a perfusion bioreactor process. The cell densities achieved in perfusion culture (30–100 million cells/mL) are typically higher than for fed-batch modes (5−25 million cells/mL). These improvements have led to lower contamination in the harvest and better yields without significant increase in cost. A perfusion bioreactor may use a cell retention device to prevent escape of the culture when byproducts are being removed. These cell retention systems add a level of complexity to the perfusion process, which may be seen in the additional management, control, and/or maintenance activities that can lead to successful operation. Operational issues such as malfunction or failure of the cell retention equipment has previously been a problem with perfusion bioreactors, which has limited their attractiveness in the past.

BRIEF DESCRIPTION

An aspect of the present disclosure provides a process for separating biomolecules from a fluid mixture. The process comprises flowing a fluid mixture containing biomolecules and cells through an acoustic perfusion device. The acoustic perfusion device comprises an acoustic chamber that receives the fluid mixture containing the biomolecules and cells, and an ultrasonic transducer that includes a piezoelectric material that can be excited to generate a multi-dimensional acoustic standing wave in the acoustic chamber. The process further comprises exciting the ultrasonic transducer to create the multi-dimensional acoustic standing wave, and modulating the frequency of the multi-dimensional acoustic standing wave to retain cells in a first size range and pass cells in a second, smaller size range; and returning the cells in the first size range to a bioreactor to thereby increase productivity of the bioreactor.

Another aspect of the present disclosure provides a process for collecting biomolecules from a cell culture. The process comprises flowing a nutrient fluid stream through the cell culture to collect the biomolecules; flowing the nutrient fluid stream through an acoustic perfusion device, the acoustic perfusion device including at least one ultrasonic transducer including a piezoelectric material configured to generate a multi-dimensional acoustic standing wave that holds cells in a first size range and passes cells in a second, smaller size range of the cell culture in the acoustic perfusion device; driving the ultrasonic transducer to generate the multidimensional acoustic standing wave and separate any biomolecule aggregates present in the nutrient fluid stream; and separating the biomolecules from the nutrient fluid stream; and returning the cells in the first size range to a bioreactor to thereby increase productivity of the bioreactor.

Another aspect of the present disclosure provides a process for separating biomolecules from a fluid mixture. The process comprises flowing a fluid mixture containing biomolecules and cells through an acoustic perfusion device. The acoustic perfusion device comprises an acoustic chamber through which the fluid mixture containing the biomolecules and cells flows, and an ultrasonic transducer and a reflector opposite the ultrasonic transducer, the ultrasonic transducer including a piezoelectric material driven

to create a multi-dimensional acoustic standing wave in the acoustic chamber. The process further comprises driving the ultrasonic transducer to create the multi-dimensional acoustic standing wave to retain cells in a first size range and pass cells in a second, smaller size range. The cell specific perfusion rate (CSPR) is improved over tangential flow filtration or alternating tangential flow filtration, or the titer of the monoclonal antibody or recombinant protein from the bioreactor to the harvest stream after acoustic separation remains near 100%; and returning the cells in the first size range to a bioreactor to thereby increase productivity of the bioreactor.

Also disclosed are biomolecules produced by one of the processes as described.

Another embodiment of the present disclosure provides an acoustic perfusion device, comprising an acoustic chamber, and an ultrasonic transducer including a piezoelectric material and configured to create a multi-dimensional acoustic standing wave in the acoustic chamber. The acoustic chamber is made of a gamma-stable material.

[0004] The present disclosure relates, in various embodiments, to acoustic devices and processes which are used for perfusion biomanufacturing. More particularly, the

CLAIMS:

1. A process for separating biomolecules from a fluid mixture, the process comprising:

flowing a fluid mixture containing biomolecules and cells through an acoustic perfusion device, the acoustic perfusion device comprising:

an acoustic chamber that receives the fluid mixture containing the biomolecules and cells; and

an ultrasonic transducer that includes a piezoelectric material that can be excited to generate a multi-dimensional acoustic standing wave in the acoustic chamber;

exciting the ultrasonic transducer to create the multi-dimensional acoustic standing wave; ~~and~~

modulating the frequency of the multi-dimensional acoustic standing wave to retain cells in a first size range and pass cells in a second, smaller size range; and

returning the cells in the first size range to a bioreactor to thereby increase productivity of the bioreactor.

2. The process of claim 1, wherein the cells in the first size range are trapped by the multi-dimensional acoustic standing wave and the cells in the second size range and biomolecules pass through the multi-dimensional acoustic standing wave.

3. The process of claim 2, wherein the trapped cells are recycled to a bioreactor upstream of the acoustic perfusion device.

4. The process of claim 1, wherein the biomolecules are therapeutic antibodies.

5. The process of claim 4, wherein a fucosylation of the therapeutic antibodies is increased and the efficacy of the therapeutic antibodies is changed.

6. The process of claim 1, wherein the biomolecules are produced by culturing cells in a bioreactor prior to flowing the fluid mixture through the acoustic perfusion device.

7. The process of claim 1, wherein a pressure rise and an acoustic radiation force on cells are generated at an interface region of the multi-dimensional acoustic standing wave to clarify the fluid mixture as it passes through the multi-dimensional acoustic standing wave.

8. The process of claim 1, wherein the acoustic perfusion device further comprises a recirculating fluid stream that transports away cells that are held back at the interface region of the multi-dimensional acoustic standing wave.

9. The process of claim 1, wherein the multi-dimensional acoustic standing wave results in an acoustic radiation force that includes an axial force component and a lateral force component that are of the same order of magnitude.

10. The process of claim 1, wherein biomolecule aggregates that pass through the multi-dimensional acoustic standing wave are collected outside and downstream of the acoustic perfusion device.

11. The process of claim 1, wherein biomolecules recovered from the acoustic perfusion device are subjected to further processing downstream of the acoustic perfusion device.

12. The process of claim 11, wherein the further processing includes at least one of chromatography and additional filtration.

13. The process of claim 12, wherein the additional filtration includes at least one of depth filtration, crossflow filtration, tangential filtration, and sterile filtration.

14. The process of claim 1, further comprising glycoengineering the biomolecules to produce antibodies with predetermined glycoforms.

15. The biomolecules produced by the process of claim 1.

16. A process for collecting biomolecules from a cell culture, the process comprising:

flowing a nutrient fluid stream through the cell culture to collect the biomolecules;

flowing the nutrient fluid stream through an acoustic perfusion device, the acoustic perfusion device including at least one ultrasonic transducer including a piezoelectric material configured to generate a multi-dimensional acoustic standing wave that holds cells in a first size range and passes cells in a second, smaller size range of the cell culture in the acoustic perfusion device; ~~and~~

driving the ultrasonic transducer to generate the multi-dimensional acoustic standing wave and separate any biomolecule aggregates present in the nutrient fluid stream; ~~and~~

separating the biomolecules from the nutrient fluid stream; and

returning the cells in the first size range to a bioreactor housing the cell culture to thereby increase productivity of the bioreactor.

17. The process of claim 16, wherein the biomolecules are monoclonal antibodies.

18. The process of claim 16, wherein any cells in the nutrient fluid stream are recycled back to the cell culture.

19. The biomolecules produced by the process of claim 16.

20. A process for separating biomolecules from a fluid mixture, the process comprising:

flowing a fluid mixture containing biomolecules and cells through an acoustic perfusion device, the acoustic perfusion device comprising:

an acoustic chamber through which the fluid mixture containing the biomolecules and cells flows; and

an ultrasonic transducer and a reflector opposite the ultrasonic transducer, the ultrasonic transducer including a piezoelectric material driven to create a multi-dimensional acoustic standing wave in the acoustic chamber; and

driving the ultrasonic transducer to create the multi-dimensional acoustic standing wave to retain cells in a first size range and pass cells in a second, smaller size range;

where the cell specific perfusion rate (CSPR) is improved over tangential flow filtration or alternating tangential flow filtration, or where the titer of the monoclonal antibody or recombinant protein from the bioreactor to the harvest stream after acoustic separation remains near 100%; and

returning the cells in the first size range to a bioreactor to thereby increase productivity of the bioreactor.