have been abandoned because of their scalability limitations or lack of proven market acceptance.

[0012] Accordingly, a need exists for improved retention devices and filters that overcome the problems discussed above.

BRIEF SUMMARY

[0013] The subject technology is based in part on the surprising discovery that large pore size (~ 5 - 10 μm) hollow-fiber filters operating in a TFF mode solve the fouling problems in perfusion culture systems, and operate efficiently and consistently for up to 35 days without significantly losing their product sieving capabilities. The cell retention devices and methods of the subject technology achieve high product sieving by TFF without a need for special treatment (e.g., gas scrubbing), without a need for a special design to increase pressure gradient across the filter (including the application of back pressure or increasing shear or flow rate), without a need to change the perfusion rate, without a need maintaining a particular perfusion volume in relation to the volume of the cell retention device, or without a need for treating the cell culture fluid (bioreactor contents) with a particular composition (e.g., a composition containing oxygen) before circulating the cell culture fluid through the cell retention device of the subject technology.

[0014] The subject technology is illustrated, for example, according to various aspects described below.

[0015] The subject technology relates to a cell retention device containing a hollow-fiber filter having an average pore size ranging from about 0.5 to about 20 μm. In an aspect, the subject technology relates to a cell retention device comprising a hollow-fiber filter having an average pore size ranging from 5 to 8 μm, wherein the cell retention device is configured to operate in conjunction with a perfusion culture vessel, ~~and~~ wherein the hollow-fiber filter has filter capacity of 1000 to 10,000 L/M^2, wherein the cells have a diameter of 10 to 15 μm, and wherein the filter capacity in L/M^2 is determined by the following steps: (1) cultivating the cells of a cell line in a perfusion cell culture vessel in tangential-flow filtration (TFF) perfusion mode equipped with a cell retention device containing the hollow-fiber filter with a defined surface area, (2) measuring the volume of the perfusion cell culture medium filtered through the cell retention device throughout the culture of step (1) until filter fouling is observed and

(3) dividing the filtered volume of the perfusion cell culture medium obtained from step (2) by the surface area of the hollow-fiber filter defined in step (1), wherein the so measured filter capacity in L/M^2 is specific for the cell line chosen for (1) and the applied culture and filtration conditions of steps (1) and (2). In one or more ~~embodiments~~examples, directly or indirectly, related to this aspect: the cell retention device is external to a perfusion cell culture vessel; the hollow-fiber filter has an average pore size selected from about 5 to about 8 µm, 5 µm to 15 µm, 5.2 µm to 12 µm, 5.5 µm to 8 µm, 5.2 µm to 7.7 µm, 5 µm, 6 µm, 7 µm, 8 µm, 9 µm, 10 µm, 11 µm, 12 µm, 13 µm, 15 µm, or any size above 5 µm that could retain the viable cells while allowing dead cells, cellular debris or macromolecules to pass through the filter; the hollow-fiber filter has an average pore size that is equal or greater than 5 µm but less than the size of viable cells being cultured in a cell culture

vessel; the hollow-fiber filter has an average pore size greater than the size of a viable cell by 5 µm or less; the cell retention device is configured to operate by tangential-flow filtration (TFF) or by alternating tangential flow filtration (ATF); the TTF or ATF are configured to operate under a low shear rate of about 1000 s-1 to about 4000 s-1; the hollow-fiber filter has filter capacity of about 1000 to about 10,000 L/M2; the cell retention device is configured to operate in conjunction with a perfusion culture vessel; the hollow-fiber filter is made out of ceramic, polymer or metallic materials; the hollow-fiber filter operates for up to 35 days without being clogged or losing more than 10% of its product sieving ability; the hollow-fiber filter operates in TTF or ATF modes under a low shear rate of 2000 s-1 or less or a perfusion flow rate of equal or greater than 4000 L/M2; the hollow-fiber filter is in a housing that facilitates the collection of filtrates.

[0016] The subject technology relates to a method for harvesting recombinant protein products from a perfusion culture vessel, said method including the steps of: (a) subjecting cell culture fluid of a perfusion culture vessel to a cell retention device to be filtered, wherein the cell culture fluid comprises cells and recombinant protein products produced by said cells and wherein the cell retention device comprises a hollow-fiber filter having an average pore size ranging from about 0.5 to about 20 µm; (b) collecting filtrate from the cell retention device, wherein the filtrate comprises the recombinant protein products; and (c) Recirculating the filtered cell culture fluid to the perfusion culture vessel. In another aspect, the subject technology relates to a method for harvesting recombinant protein products from a perfusion culture vessel, said method including the steps of: (a) subjecting cell culture fluid of a perfusion culture vessel to a cell retention device to be filtered, wherein the cell culture fluid comprises cells and recombinant protein products produced by said cells and wherein the cell retention device comprises a hollow-fiber filter having an average pore size ranging from 5 to 8 µm, and wherein the hollow-fiber filter has filter capacity of 1000 to 10,000 L/M^2; (b) collecting filtrate from the cell retention device, wherein the filtrate comprises the recombinant protein products; and (c) Recirculating the filtered cell culture fluid to the perfusion culture vessel, wherein the cells have a diameter of 10 to 15 µm, and wherein the filter capacity in L/M^2 is determined by the following steps: (1) cultivating the cells of a cell line in a perfusion cell culture vessel in TFF perfusion mode equipped with a cell retention device containing the hollow-fiber filter with a defined

surface area, (2) measuring the volume of the perfusion cell culture medium filtered through the cell retention device throughout the culture of step (1) until filter fouling is observed and (3) dividing the filtered volume of the perfusion cell culture medium obtained from step (2) by the surface area of the hollow-fiber filter defined in step (1), wherein the so measured filter capacity in L/M^2 is specific for the cell line chosen for (1) and the applied culture and filtration conditions of steps (1) and (2). In one or more ~~embodiments~~examples, directly or indirectly, related to this aspect: the cell retention device is external to the perfusion cell culture vessel; the hollow-fiber filter filters the cell culture fluid by tangential-flow filtration (TFF) or alternating tangential flow filtration (ATF); the hollow-fiber filter has an average pore size selected from about 5 to about 8 micron, 5 µm to 15 µm, 5.2 µm to 12 µm, 5.5 µm to 8 µm, 5.2 µm to 7.7 µm, 5 µm, 6 µm, 7 µm, 8 µm, 9 µm, 10 µm, 11 µm, 12 µm, 13 µm, 15 µm, or any size above 5 µm that could retain the viable cells while allowing dead cells, cellular debris or macromolecules to pass through the filter; the hollow-fiber filter has an average pore size that is equal or greater than 5 micron but less than the size of the cells; the hollow-fiber filter has an average pore size greater than the size of the cells by 5 µm or less; the hollow-fiber filter has an average pore size that is about the size of the cells; the hollow-fiber filter is made out of ceramic, metallic or polymer materials; the filtrate comprises the recombinant protein products but lacks viable cells; the hollow-fiber filter operates for up to 35 days without being

clogged or losing more than 10% of its product sieving ability; the cell retention device operates in TTF or ATF modes under a low shear rate of about 1000 s-1 to about 4000 s-1; the hollow-fiber filter has filter capacity of about 1000 to about 10,000 L/M2. ~~In another embodiment~~Where mentioned above, the subject technology relates to a protein made according to this aspect or to any of its ~~embodiments~~examples.

[0017] The subject technology relates to a perfusion culture system, including: (a) a perfusion culture vessel configured to contain a cell culture fluid, wherein the cell culture fluid comprises liquid media, cells and recombinant protein products produced by said cells; (b) a cell retention device configured to receive the cell culture fluid and filter same to provide a filtrate comprising recombinant protein products, wherein the cell retention device comprises a hollow-fiber filter having an average pore size ranging from about 0.5 to about 20 µm; and (c) a pump and a fluid connector to circulate the cell culture fluid from the perfusion culture vessel to the cell retention device and back to the perfusion culture vessel. In another aspect, the subject technology relates to a perfusion culture system, including: (a) a perfusion culture vessel configured to contain a cell culture fluid, wherein the cell culture fluid comprises liquid media, cells and recombinant protein products produced by said cells; (b) a cell retention device configured to receive the cell culture fluid and filter same to provide a filtrate comprising recombinant protein products, wherein the cell retention device comprises a hollow-fiber filter having an average pore size ranging from 5 to 8 µm, and wherein the hollow-fiber filter has filter capacity of 1000 to 10,000 L/M^2; and (c) a pump and a fluid connector to circulate the cell culture fluid from the perfusion culture vessel to the cell retention device and back to the perfusion culture vessel, wherein the cells have a diameter of 10 to 15 µm, and wherein the filter capacity in L/M^2 is determined by the following steps: (1) cultivating the cells of a cell line in a perfusion cell culture vessel in TFF perfusion mode equipped with a cell retention device containing the hollow-fiber filter with a defined surface area, (2) measuring the volume of the perfusion cell culture medium filtered through the cell retention device throughout the culture of step (1) until filter fouling is observed and (3) dividing the filtered volume of the perfusion cell culture medium obtained from step (2) by the surface area of the hollow-fiber filter defined in step (1), wherein the so measured filter capacity in L/M^2 is specific for the cell line chosen for (1) and the

applied culture and filtration conditions of steps (1) and (2). In one or more ~~embodiments~~examples, directly or indirectly, related to this aspect: the cell retention device is external to the perfusion cell culture vessel; the cell retention device operates by tangential-flow filtration (TFF) or alternating tangential flow filtration (ATF); the hollow-fiber filter has an average pore size selected from about 5 to about 8 micron, 5 µm to 15 µm, 5.2 µm to 12 µm, 5.5 µm to 8 µm, 5.2 µm to 7.7 µm, 5 µm, 6 µm, 7 µm, 8 µm, 9 µm, 10 µm, 11 µm, 12 µm, 13 µm, 15 µm, or any size above 5 µm that could retain the viable cells while allowing dead cells, cellular debris or macromolecules to pass through the filter; the hollow-fiber filter has an average pore size that is equal or greater than 5 micron but smaller than the size of the cells; the hollow-fiber filter has an average pore size greater than the size of the cells by 5 µm or less; the hollow-fiber filter has an average pore size that is about the size of the cells; the filtrate comprises the recombinant protein products but lacks viable cells; the hollow-fiber filter is made out of ceramic, metallic or polymer materials or combination thereof; the hollow-fiber filter operates for up to about 35 days without being clogged or losing more than 10% of its product sieving ability; the cell retention device operates in TTF or ATF modes under a low shear rate of about 1000 s-1 to about 4000 s-1; the hollow-fiber filter has filter capacity of about 1000 to about 10,000 L/M2; the hollow-fiber filter allows the passage of macromolecules with a molecular weight of equal to or greater than 50 kD after 5 days of use without losing more than 10% of its product sieving ability; the hollow-fiber filter allows the passage of macromolecules

CLAIMS

1. A cell retention device comprising a hollow-fiber filter having an average pore size ranging from 5 to 8 µm,

   wherein the cell retention device is configured to operate in conjunction with a perfusion culture vessel, ~~and~~ wherein the hollow-fiber filter has filter capacity of 1000 to 10,000 L/M^2, wherein the cells have a diameter of 10 to 15 µm, and wherein the filter capacity in L/M^2 is determined by the following steps:

   (1) cultivating the cells of a cell line in a perfusion cell culture vessel in tangential-flow filtration (TFF) perfusion mode equipped with a cell retention device containing the hollow-fiber filter with a defined surface area,

   (2) measuring the volume of the perfusion cell culture medium filtered through the cell retention device throughout the culture of step (1) until filter fouling is observed and

   (3) dividing the filtered volume of the perfusion cell culture medium obtained from step (2) by the surface area of the hollow-fiber filter defined in step (1), wherein the so measured filter capacity in L/M^2 is specific for the cell line chosen for (1) and the applied culture and filtration conditions of steps (1) and (2).

2. The cell retention device of claim 1, wherein the cell retention device is external to a perfusion cell culture vessel.

3. The cell retention device of one or more of claims 1 to 2, wherein the hollow-fiber filter has an average pore size selected from 5.5 µm to 8 µm, 5.2 µm to 7.7 µm, 5 µm, 6 µm, 7 µm, 8 µm, or any size above 5 µm that could retain the viable cells while allowing dead cells, cellular debris or macromolecules to pass through the filter.

~~4. The cell retention device of one or more of claims 1 to 3, wherein the hollow-fiber filter has an average pore size that is less than the size of viable cells being cultured in a cell culture vessel.~~

~~5. The cell retention device of one or more of claims 1 to 3, wherein the hollow-fiber filter has an average pore size greater than the size of a viable cell by 5 µm or less.~~

~~6.~~4. The cell retention device of one or more of claims 1 to ~~5~~3, wherein the cell retention device is configured to operate by ~~tangential-flow filtration (~~TFF~~)~~ or by alternating tangential flow filtration (ATF),

preferably wherein the TTF or ATF are configured to operate under a low shear rate of 1000 s^{-1} to 4000 s^{-1}.

~~7.~~5. The cell retention device of one or more of claims 1 to ~~6~~4, wherein the hollow-fiber filter is made out of ceramic, polymer or metallic materials,

preferably wherein the hollow-fiber filter operates in TTF or ATF modes under a low shear rate of 2000 s^{-1} or less or a perfusion flow rate of equal or greater than 4000 L/m^2

and/or

wherein the hollow-fiber filter is in a housing that facilitates the collection of filtrates.

~~8.~~6. The cell retention device of one or more of claims to 1 to ~~7~~5, wherein the hollow-fiber filter operates for up to 35 days without being clogged or losing more than 10% of its product sieving ability.

~~9.~~7. A method for harvesting recombinant protein products from a perfusion culture vessel, said method comprising the steps of:

a. subjecting cell culture fluid of a perfusion culture vessel to a cell retention device to be filtered, wherein the cell culture fluid comprises cells and recombinant protein products produced by said cells and wherein the cell retention device comprises a hollow-fiber filter having an average pore size ranging from 5 to 8 µm, and wherein the hollow-fiber filter has filter capacity of 1000 to 10,000 L/M^2;

b. collecting filtrate from the cell retention device, wherein the filtrate comprises the recombinant protein products; and

c. ~~Recirculating~~ recirculating the filtered cell culture fluid to the perfusion culture vessel~~.~~.

wherein the cells have a diameter of 10 to 15 µm, and wherein the filter capacity in L/M^2 is determined by the following steps:

(1) cultivating the cells of a cell line in a perfusion cell culture vessel in TFF perfusion mode equipped with a cell retention device containing the hollow-fiber filter with a defined surface area,

(2) measuring the volume of the perfusion cell culture medium filtered through the cell retention device throughout the culture of step (1) until filter fouling is observed and

(3) dividing the filtered volume of the perfusion cell culture medium obtained from step (2) by the surface area of the hollow-fiber filter defined in step (1), wherein the so measured filter capacity in L/M^2 is specific for the cell line chosen for (1) and the applied culture and filtration conditions of steps (1) and (2).

~~10.~~8. The method of claim ~~9~~7, ~~wherein the hollow-fiber filter has an average pore size that is about the size of the cells, preferably~~ wherein the filtrate comprises the recombinant protein products but lacks viable cells.

~~11.~~9. A perfusion culture system, comprising:

a. a perfusion culture vessel configured to contain a cell culture fluid, wherein the cell culture fluid comprises liquid media, cells and recombinant protein products produced by said cells;

b. a cell retention device configured to receive the cell culture fluid and filter same to provide a filtrate comprising recombinant protein products, wherein the cell retention device comprises a hollow-fiber filter having an average pore size ranging from 5 to 8 µm, and wherein the hollow-fiber filter has filter capacity of 1000 to 10,000 L/M^2; and

c. a pump and a fluid connector to circulate the cell culture fluid from the perfusion culture vessel to the cell retention device and back to the perfusion culture vessel~~.~~, wherein the cells have a diameter of 10 to 15 µm, and wherein the filter capacity in L/M^2 is determined by the following steps:

(1) cultivating the cells of a cell line in a perfusion cell culture vessel in TFF perfusion mode equipped with a cell retention device containing the hollow-fiber filter with a defined surface area,

(2) measuring the volume of the perfusion cell culture medium filtered through the cell retention device throughout the culture of step (1) until filter fouling is observed and

(3) dividing the filtered volume of the perfusion cell culture medium obtained from step (2) by the surface area of the hollow-fiber filter defined in step (1), wherein the so measured filter capacity in L/M^2 is specific for the cell line chosen for (1) and the applied culture and filtration conditions of steps (1) and (2).

~~12.~~10. The perfusion culture system of claim ~~11~~9, wherein the hollow-fiber filter allows the passage of macromolecules with a molecular weight of equal to or greater than 50 kD after 5 days of use without losing more than 10% of its product sieving ability, preferably wherein the hollow-fiber filter allows the passage of macromolecules with a molecular weight of equal to or greater than 50 kD after 10 days of use without losing more than 10% of its product sieving ability.

~~13.~~11. The perfusion culture system of claim ~~11 or 12~~9 or 10, wherein the filtrate is subjected to a Protein A column without a depth filtration step and/or wherein the filtrate is subjected to a flocculation step before being subjected to a Protein A column.

~~14.~~12. The perfusion culture system of one or more of claims ~~11 to 13~~9 to 11, wherein the cells comprise mammalian cells, plant cells, insect cells, yeast cells, or bacterial cells, preferably selected from BHK (baby Hamster kidney) cells, CHO (Chinese Hamster ovary) cells, HKB (hybrid of kidney and B cells) cells, HEK (human embryonic kidney) cells, and NSO cells.

~~15.~~13. The perfusion culture system of one or more of claims ~~11 to 14~~9 to 12, wherein the recombinant protein products are monoclonal antibodies or fragments thereof or therapeutic proteins, hormones or enzymes.