[0010a] In one aspect, there is provided a method for producing a polypeptide and/or virus of interest in a serum-free continuous cell culture, said method comprising:

(a) culturing mammalian cells expressing the polypeptide and/or virus of interest in a continuous cell culture system, wherein said cells are cultured in a serum-free medium and wherein said cell culture system comprises a cell retention device and has a dilution rate (D) of less than 0.6 d^{-1} and a specific growth rate of between 0.15 d^{-1} to 0.25 d^{-1}; and

(b) recovering said polypeptide and/or virus of interest from medium removed from said cell culture system.

[0011] Accordingly, one embodimentaspect of the disclosureinvention relates to a method for producing a polypeptide and/or virus of interest in a continuous cell culture, the method comprising culturing mammalian cells expressing the polypeptide and/or virus in a continuous cell culture system, wherein the cell culture system comprises a cell retention device and has a dilution rate of less than about 2 d^{-1} and a cell density of less than about $2X10^7$ cell/mL; and recovering the polypeptide and/or virus of interest from medium removed from the cell culture system. In some embodiments, the cells are genetically modified to recombinantly express the polypeptide and/or virus of interest.

[0012] In some embodiments, the cell retention device is chosen to be less capable of retaining cells than typical cell retention devices. In some embodiments, the cell retention device produces a cell retention rate of less than about 90%, less than about 85%, less than about 80%, or less than about 75%. In some embodiments, the cell retention device comprises a macroporous microcarrier, e.g., a cellulose-based particle.

[0013] The dilution rate and cell density preferably are maintained at chosen values or ranges. In some embodiments, the dilution rate is less than about 1 d^{-1}, e.g., between about 0.1 and about 1.0 d^{-1}. In some embodiments, the cell density is less than about $1X10^7$ cell/mL. In some embodiments, the ratio of the dilution rate and the specific growth rate (D/μ) of the cell culture system is maintained at a chosen value or range. In some preferred embodiments, the cell culture system has a ratio of the dilution rate and specific growth rate greater than about 1, for example, between about 1.2 and about 5.0, or between about 1.8 and about 3. In some embodiments, the specific growth rate is maintained between 0.2 d^{-1} and 0.8 d^{-1}.

[0014] An advantage of certain embodiments of the continuous cell culture system described herein is that the culture can be sustained for a prolonged period of time. In some embodiments, for example, the dilution rate and/or the cell density are maintained for at least about 80% of the time the cells are being cultured in the continuous cell culture system. In some embodiments, the cells are cultured in the cell culture system for more than 20 days, preferably more than 40 days, and more preferably, more than 50 days, for example, allowing increased production of the polypeptide and/or virus of interest, potentially at low cost.

[0015] Another advantage of certain embodiments of the continuous cell culture system is an increase in volumetric productivity due to higher cell density, compared to a typical chemostat culture. For example, in particular preferred embodiments, volumetric

productivity increases by 70%, 90%, or more. Still another advantage of certain embodiments of the continuous cell culture system is improved specific activity of the protein being recovered, due to for example reduced residence time in the culture unit, which may have beneficial effect on stability, structure, and/or function of the protein.

[0016] Another advantage of certain embodiments of the continuous cell culture system described herein is that the system is amenable to being scaled up for large scale productions. That is, by allowing increased cell density and/or cell growth, as compared to standard chemostat culture systems, the continuous cell culture system disclosed herein provides for commercial scale production of a polypeptide and/or virus of interest. For example, in some embodiments, the cells are cultured in at least about 250 L of medium, e.g., at least abut 500 L, or at least about 1,000 L of medium. In some preferred embodiments, the cells are cultured in serum-free media and/or chemically-defined media.

[0017] In some embodiments, the method further comprises a pre-culturing step, e.g., to adapt the cells for production of the polypeptide and/or virus of interest in a continuous cell culture system as herein described. Thus, in some preferred embodiments, the method further comprises, before the culturing step, pre-culturing the cells in suspension, e.g., until the culture reaches an appropriate volume.

[0017a] In one aspect, there is provided a method for producing a disintegrin-like and metallopeptides with thrombospondin type 1 motif 13 (ADAMTS13) protein in a continuous cell culture, said method comprising:

(a) culturing mammalian cells genetically modified to express the ADAMTS13 in a continuous cell culture system, wherein said cell culture system comprises a cell retention device that produces a cell retention rate of less than 90% and has a dilution rate (D) of less than 0.6 d^{-1}; and

(b) recovering said polypeptide and/or virus of interest from medium removed from said cell culture system.

[0018] In a particular embodiment, the polypeptide of interest is a disintegrin-like and metallopeptidase with thrombospondin type 1 motif 13 (ADAMTS13) protein. In some embodiments, the mammalian cells are CHO cells, e.g., CHO cells genetically modified to express ADAMTS13 protein. In a particular embodiment, a method is provided for producing a polypeptide and/or virus of interest in a continuous cell culture, the method comprising (a) culturing non-anchorage dependent cells mammalian cells expressing the polypeptide and/or virus of interest in a continuous cell culture system, where the cell culture system comprises a cell retention device having a cell retention rate of less than 90%, and has a dilution rate (D) between 0.1 and 1.0 d^{-1} and a cell density of less than 1 X 10^7 cell/mL; and (b) recovering the polypeptide and/or virus of interest from medium removed from the cell culture system.

[0019] In another particular embodiment, a method is provided for producing a polypeptide and/or virus of interest in a continuous cell culture, the method comprising (a) culturing non-anchorage dependent cells CHO cells expressing the polypeptide and/or virus of interest in a continuous cell culture system, where the cell culture system comprises a

macroporous microcarrier cell retention device having a cell retention rate of less than 90%, and has a dilution rate (D) between 0.1 and 1.0 d^{-1} and a cell density of less than 1 X 10^7 cell/mL; and (b) recovering the polypeptide and/or virus of interest from medium removed from the cell culture system.

[0020] In still another particular embodiment, a method is provided for producing ADAMTS13 in a continuous cell culture, the method comprising (a) culturing non-anchorage dependent cells mammalian cells expressing recombinant ADAMTS13 protein in a continuous cell culture system, where the cell culture system comprises a macroporous microcarrier cell retention device having a cell retention rate of less than 90%, and has a dilution rate (D) between 0.1 and 1.0 d^{-1} and a cell density of less than 1 X 10^7 cell/mL; and (b) recovering the ADAMTS13 from medium removed from the cell culture system.

[0021] In yet another particular embodiment, a method is provided for producing a polypeptide and/or virus of interest in a continuous cell culture, the method comprising (a) culturing non-anchorage dependent cells mammalian cells expressing the polypeptide and/or virus of interest in a continuous cell culture system, where the cell culture system comprises a cell retention device having a cell retention rate of less than 90%, and has a dilution rate (D) between 0.1 and 1.0 d^{-1}, a cell density of less than 1 X 10^7 cell/mL, and a ratio of the dilution rate and specific growth rate (D/μ) between 1.2 and 5; and (b) recovering the polypeptide and/or virus of interest from medium removed from the cell culture system; and further where the cells are cultured in the cell culture system for more than 50 days, and the dilution rate, cell density, and ratio of the dilution rate and specific growth rate each are maintained for at least 80 % of the time the cells are cultured in the cell culture system.

[0022] A further embodiment~~aspect~~ of the disclosure~~invention~~ relates to a composition comprising a polypeptide and/or virus of interest produced according to methods described herein, e.g., a composition comprising a recombinant ADAMTS13 protein produced accordingly.

[0023] These and other aspects of the invention are described in more detail below.

DETAILED DESCRIPTION

[0024] The continuous cell culture system as described herein combines some of the advantages of both perfusion culture and chemostat culture of mammalian cells. As described above, perfusion culture systems are operated with fresh medium perfusing through the cell culture, while the cells are retained using a cell retention device; chemostat culture