[020] In view of the challenges with perfusion cell culture noted in the art, additional media supplements or additives that are effective in suppressing cell growth in the perfusion state and avoid the need for cell bleeding would significantly help advance the art.

SUMMARY

[021] The present disclosure relates in part to the discovery that certain lipids and lipid metabolites and/or derivatives and/or precursors thereof when included singularly or in combination in cell culture media, e.g., linoleic acid, arachidonic acid, and prostaglandin E2, or derivatives and/or precursors thereof, are effective at causing an increase in cell specific productivity and/or are effective in maintaining or sustaining high cell viability and/or suppressing cell growth of the cell culture. These agents can be used in connection with any type of cell culture system, including batch, fed-batch, and continuous perfusion cell culture systems. In a particular example, the cell culture system is a continuous perfusion cell culture system.

[022] It was found that the lipids and lipid metabolites described herein, e.g., linoleic acid, arachidonic acid, and prostaglandin E2, or derivative and/or precursors thereof, were effective in suppressing cell growth, and that this growth suppression led to an increase in cell specific productivity and helped in maintaining high viability in a cell culture.

[023] With regard to perfusion cell cultures, the cell growth suppression by the lipids and/or lipid metabolites described herein (e.g., linoleic acid, arachidonic acid, and prostaglandin E2, or derivatives and/or precursors thereof) not only led to an increase in cell specific productivity and sustained high cell viability in the perfusion cell culture, while reducing or eliminating the need to employ cell bleeding techniques during the perfusion state to otherwise maintain the cells in a steady state of growth. In other words, the suppression of cell growth in the perfusion state by the lipids and lipid metabolites of the disclosure increases overall cell specific productivity and helps to

maintain high cell viability while minimizing or eliminating wasteful and undesirable cell bleeding techniques.

[024] As indicated above, a wasteful cell bleed can be utilized to maintain a sustainable viable cell density and preserve viability in connection with continuous cell culturing processes. In continuous processes, a large proportion of the culture medium and hence product can be lost due to the technique of cell bleeding, which siphons off proliferating cells and medium in order to maintain a constant, sustainable viable cell density within the bioreactor. Up to one-third of harvestable material can be lost due to cell bleeding techniques. Using cell bleeding therefore decreases the product yield per run as the product within the portion removed by cell bleeding is not harvested. In order to decrease the volume of culture removed by cell bleeding and thus retain more supernatant for harvesting it is advantageous to inhibit cell proliferation once the desired viable cell density is reached in the production phase. There is a need for controlling cell growth once an optimal variable cell density has been obtained, without utilizing wasteful cell bleeding techniques, thereby increasing product recovered per perfusion run and generating a more efficient method for operating perfusion processes.

[025] As demonstrated in the specific embodiments of the Examples herein, the present disclosure provides a perfusion culture medium comprising one or more of linoleic acid, arachidonic acid, or prostaglandin E2. The disclosure further provides methods of culturing mammalian cells, e.g., Chinese Hamster Ovary (CHO) cells, using said medium are provided. In certain examples, the cell culturing method can be fed-batch or batch methods. In other examples, the cell culturing method can be a continuous perfusion culturing method. The Examples demonstrate that linoleic acid, arachidonic acid, and prostaglandin E2—alone or in combination—have the same effect on mammalian cells in cell culture, e.g., perfusion cell culture, wherein said effect is growth suppression with a concomitant increase in production of product, without the need for wasteful cell bleeding.

[026] Thus, the following aspects of the disclosure are provided herein.

[026a] In a first aspect of the disclosure, a method of culturing mammalian cells expressing a heterologous protein in a perfusion cell culture is provided comprising: culturing the mammalian cells in a culture medium comprising an effective amount of one or more lipids or lipid metabolites, wherein the one or more lipids or lipid metabolites comprises 500-2000 µM linoleic acid, 100-600 µM arachidonic acid and/or 0.0001-100 µM prostaglandin E2.

[026b] In a second aspect of the disclosure, a method of producing a therapeutic protein from a perfusion cell culture is provided comprising: (a) culturing mammalian cells expressing a heterologous protein in a culture medium comprising an effective amount of one or more lipids or lipid metabolites, wherein the one or more lipids or lipid metabolites comprises 500-2000 µM linoleic acid, 100-600 µM arachidonic acid and/or 0.0001-100 µM prostaglandin E2, (b) harvesting the heterologous protein from the perfusion cell culture.

[026c] In a third aspect of the disclosure, a perfusion cell culture medium is provided comprising one or more lipids or lipid metabolites comprising 500-2000 µM linoleic acid, 100-600 µM arachidonic acid and/or 0.0001-100 µM prostaglandin E2.

[026d] In a fourth aspect of the disclosure, a use of the perfusion cell culture medium as defined herein for culturing mammalian cells in a perfusion culture is provided.

[026e] In a fifth aspect of the disclosure, a use of the perfusion cell culture medium as defined herein for suppressing the growth of the perfusion culture and increasing productivity is provided.

[027] In the disclosure, a method of culturing mammalian cells expressing a heterologous protein in a cell culture is provided comprising culturing

mammalian cells expressing a heterologous protein in a culture medium comprising an effective amount of one or more lipids or lipid metabolites selected from the group consisting of: linoleic acid, arachidonic acid, and prostaglandin E2, or derivatives and/or precursors thereof. In certain examples, the lipid or lipid metabolite or combination thereof results in growth suppression and/or increased cell specific productivity. In certain other examples relating to perfusion cell culture, the growth suppression and/or increased cell specific productivity may reduce or eliminate the need for cell bleeding to maintain or achieve a steady state.

[028] In the disclosure, a method of culturing mammalian cells expressing a heterologous protein in a perfusion cell culture is provided comprising culturing mammalian cells expressing a heterologous protein in a perfusion culture medium comprising one or more of an effective amount of a lipid or lipid metabolite selected from the group consisting of: linoleic acid, arachidonic acid, and prostaglandin E2, or derivatives and/or precursors thereof.

[029] In the disclosure, a method of culturing mammalian cells expressing a heterologous protein in a perfusion cell culture is provided comprising culturing mammalian cells expressing a heterologous protein in a perfusion culture medium comprising an effective amount of linoleic acid.

[030] In the disclosure, a method of culturing mammalian cells expressing a heterologous protein in a perfusion cell culture is provided comprising culturing mammalian cells expressing a heterologous protein in a perfusion culture medium comprising an effective amount of arachidonic acid.

[031] In the disclosure, a method of culturing mammalian cells expressing a heterologous protein in a perfusion cell culture is provided comprising culturing mammalian cells expressing a heterologous protein in a perfusion culture medium comprising an effective amount of prostaglandin E2.

[032] In the disclosure, a method of enhancing the specific productivity of a perfusion cell culture is provided comprising (a) culturing recombinant mammalian cells encoding a protein of interest in a perfusion growth medium until a desired cell density is reached, and (b) introducing an effective amount of one or more

of a lipid or lipid metabolite selected from the group consisting of: linoleic acid, arachidonic acid, and prostaglandin E2, or derivatives and/or precursors thereof, into the growth medium., In certain examples, the lipids or lipid metabolites or combination thereof results in growth suppression and/or increased cell specific productivity. In certain other examples relating to perfusion cell culture, the growth suppression and/or increased cell specific productivity may reduce or eliminate the need for cell bleeding to maintain or achieve a steady state.

[033] In the disclosure, a cell culture medium for culturing mammalian cells and producing therapeutic proteins with improved productivity is provided. The cell culture medium comprises one or more of an effective amount of one or more lipids or lipid metabolites selected from the group consisting of: linoleic acid, arachidonic acid, and prostaglandin E2, or derivatives and/or precursors thereof. In certain examples, the lipids or lipid metabolites or combination thereof results in growth suppression and/or increased cell specific productivity. In certain other examples relating to perfusion cell culture, the growth suppression and/or increased cell specific productivity may reduce or eliminate the need for cell bleeding to maintain or achieve a steady state. The cell culture medium can be used in any cell culture platform, including batch cultures, fed-batch cultures, and continuous cultures, including continuous perfusion cultures.

[034] In various examples, the cell culture is not particularly limited and may encompass all forms and techniques of cell culture, including but not limited to, fed-batch, batch, perfusion, continuous, finite, suspension, adherent or monolayer, anchorage-dependent, and 3D cultures. Any configuration of cell culture is contemplated herein. In a preferred example, the cell culture is a continuous perfusion cell culture.

[035] In various examples, the mammalian cells grown in cell culture are Chinese Hamster Ovary (CHO) cells. In various other examples, the mammalian cells are not particularly limited. They can include any commonly used cell lines from hamster (e.g., CHO or CHO-S cells), human (e.g., Jurkat cells, 293 cells, HeLa cells), monkey (e.g., CV-1 cells), or mouse (e.g., 3T3 cells). The cells can be from any tissue,

for example ovary cells (e.g., CHO of CHO-S) or kidney cells (e.g., 293 cells). The cells used in any of the methods herein may also encompass any cells that may be obtained from commercial sources, e.g., THERMOFISHER SCIENTIFIC®.

[036] In various examples, the effective amount of linoleic acid is from about 1800-2000 µM. In other examples, the effective amount of linoleic acid is from about 1-5000 µM, or from about 1-2500 µM, or from about 1-1250 µM, or from about 1-1000 µM, or from about 1-800 µM, or from about 1-600 µM, or from about 1-400 µM, or from about 1-200 µM, or from about 1-100 µM, or from about 1-50 µM, or about 1-25 µM. In one example, the linoleic acid is in a concentration of 500-2000 µM.

[037] In other examples, the effective amount of arachidonic acid is from about 150-500 µM. In still other examples, the effective amount of arachidonic acid is from about 1-5000 µM, or from about 1-2500 µM, or from about 1-1250 µM, or from about 1-1000 µM, or from about 1-800 µM, or from about 1-600 µM, or from about 1-400 µM, or from about 1-200 µM, or from about 1-100 µM, or from about 1-50 µM, or about 1-25 µM. In one example, the arachidonic acid is in a concentration of 100-600 µM.

[038] In other examples, the effective amount of prostaglandin E2 is from about 0.001 µM to 60 µM. In still other examples, the effective amount of prostaglandin E2 is 0.000001 µM or more, 0.00001 µM or more, 0.0001 µM or more, 0.001 µM or more, 0.01 µM or more, 0.1 µM or more, 1 µM or more, 10 µM or more, 20 µM or more, 30 µM or more, 40 µM or more, 50 µM or more, 60 µM or more, 70 µM or more, 80 µM or more, 90 µM or more, 100 µM or more, 150 µM or more, 200 µM or more, wherein the upper limit of each range may be 1000 µM or less. In yet other examples, the effective amount of prostaglandin E2 is about 1-5000 µM, or from about 1-2500 µM, or from about 1-1250 µM, or from about 1-1000 µM, or from about 1-800 µM, or from about 1-600 µM, or from about 1-400 µM, or from about 1-200 µM, or from about 1-100 µM, or from about 1-50 µM, or about 1-25 µM, or from about 0.0001-0.001 µM, to about 0.0005-0.01 µM, to about 0.005-0.1 µM, to about 0.05-1.0

μM to about 0.5-100 μM, to about 50-1000 μM or more. In one example, the prostaglandin E2 is in a concentration of 0.0001-100 μM.

[039] In some examples, the culture medium comprises at least two of the lipids or lipid metabolites. In one example, the culture medium comprises arachidonic acid

at a concentration of 100-300 µM and linoleic acid at a concentration of 500-1800 µM. In another example, the culture medium comprises prostaglandin E2 at a concentration of 0.0001-0.0009 µM in combination with either linoleic acid at a concentration of 500-1800 µM or arachidonic acid at a concentration of 100-150 µM.

[040] In still another example, the culture medium comprises all three lipids or lipid metabolites. In one example, the culture medium comprises linoleic acid in a concentration of 500-2000 µM, the arachidonic acid at a concentration of 100-300 µM, and the prostaglandin E2 at a concentration of 0.0001-0.0009 µM.

[041] In various examples, the heterologous protein is not particularly limited, and can be, for example, a therapeutic protein, such as, but not limited to an antibody, a fusion protein, a cytokine, or a growth factor, or a fragment thereof. The antibodies can be monoclonal antibodies and can be in any alternative molecular format, including (a) antigen-binding building blocks (e.g., single variable domain binding sites; two variable domain binding sites; diabody), (b) bispecific antibody fragments (e.g., tandem scFv, tandem scFv-Fc, scFv-Fc knobs-into-holes, scFv-Fc-scFv, $F(ab')_2$, Fab-scFv, $(Fab'scFv)_2$, diabody, scDiabody, scDiabody-Fc, scDiabody-C_H3), (c) IgG-based bispecific antibodies (e.g., hybrid hybridoma, knobs-into-holes with common light chain, two-in-one IgG, dual V domain IgG, IgG-scFv, scFv-IgG, IgG-V, and V-IgG). See Chan et al., Nature Reviews Immunology, Vol. 10, pp. 301-316 (May 2010), incorporated herein by reference.

[042] In various examples, the one or more lipids or lipid metabolites are added or initiated only when the cells in the cell culture reach a certain density. In certain embodiments, the cell density is 10 x 10^6 cells/ml to about 120 x 10^6 cells/ml or even higher. The cell density for lipid initiation may also be at least 10 x 10^6 cells/ml, at least 20 x 10^6 cells/ml, at least 30 x 10^6 cells/ml, at least 40 x 10^6 cells/ml or at least 50 x 10^6 cells.

[043] In certain examples, a method is provided for culturing mammalian cells during a growth phase by perfusion with a serum-free perfusion medium until the cells reach a desired viable cell density; and then growing the cells during a production phase in a serum-free perfusion medium comprising one or more of a lipid or lipid metabolite

selected from the group consisting of linoleic acid, arachidonic acid, and prostaglandin E2, or effective derivatives and/or precursors thereof. In certain examples, the arachidonic acid, if present, is at a concentration of 100-300 µM, the linoleic acid, if present, is at a concentration of 500-1800 µM, and the prostaglandin E2, if present, is at a concentration of 0.0001-0.0009 µM. In certain other examples, the growth phase continues until the desired viable cell density (VCD) reaches 10 x 10^6 cells/ml to about 120 x 10^6 cells/ml or even higher, or reaches about 10 x 10^6 cells/ml, or about 20 x 10^6 cells/ml, or about 30 x 10^6 cells/ml, or about 40 x 10^6 cells/ml or about 50 x 10^6 cells.

[044] In certain example pertaining to perfusion cell culture, the method of cell culture may include a further step of cell bleeding. However, the use of cell bleeding may be reduced or eliminated with the lipid-containing media of the herein described methods compared to a perfusion cell culture using the same perfusion medium but without including the lipids or lipid metabolites described herein.

[045] The osmolarity of the perfusion medium can be in the range of between 300 and 1400 mOsmol/kg, preferably between 300 and 500 mOsmol/kg, more preferably between 330 and 450 mOsmol/kg and even more preferably between 360 and 390 mOsmol/kg.

[046] The perfusion medium may be serum-free, and it may further be chemically defined and/or hydrolysate free. Preferably the perfusion medium can be protein-free or protein-free except for recombinant insulin and/or insulin-like growth factor, more preferably the serum-free perfusion medium is chemically defined and protein-free or protein-free except for recombinant insulin and/or insulin-like growth factor.

[047] In a further example a method of producing a therapeutic protein using the methods of the disclosure is provided optionally comprising a further step of purifying and formulating the therapeutic protein into a pharmaceutically acceptable formulation.

[048] In particular further examples, the present disclosure provides the following examples which are not in any way intended to limit the scope of the herewith disclosure.

[049] In one example, the disclosure provides a method of culturing mammalian cells expressing a heterologous protein in a cell culture, comprising: culturing

mammalian cells expressing a heterologous protein in a culture medium comprising an effective amount of one or more lipids or lipid metabolites selected from the group consisting of: linoleic acid, arachidonic acid, and prostaglandin E2, or derivatives and/or precursors thereof. In certain examples, the lipids or lipid metabolites or combination thereof results in growth suppression and/or increased cell specific productivity. In certain other examples relating to perfusion cell culture, the growth suppression and/or increased cell specific productivity may reduce or eliminate the need for cell bleeding to maintain or achieve a steady state.

[050] In another example, the disclosure provides a method of producing a therapeutic protein from a cell culture, comprising:

(a) culturing mammalian cells expressing a heterologous protein in a culture medium comprising an effective amount of one or more lipids or lipid metabolites selected from the group consisting of: linoleic acid, arachidonic acid, and prostaglandin E2, or derivatives and/or precursors thereof,

(b) harvesting the heterologous protein from the cell culture.

[051] In certain examples, the lipids or lipid metabolites or combination thereof results in growth suppression and/or increased cell specific productivity. In certain other examples relating to perfusion cell culture, the growth suppression and/or increased cell specific productivity may reduce or eliminate the need for cell bleeding to maintain or achieve a steady state.

[052] In various examples, the perfusion cell culture medium comprises one or more lipids or lipid metabolites selected from the group consisting of linoleic acid, arachidonic acid, and prostaglandin E2, and functionally equivalent derivatives and/or precursors thereof.

[053] In various examples, the linoleic acid or derivative thereof is in a concentration of 500-2000 µM.

[054] In various examples, the arachidonic acid or derivative thereof is in a concentration of 100-600 µM.

[055] In various examples, the prostaglandin E2 or derivative thereof is in a concentration of 0.0001-100 µM.

[056] In various examples, the culture medium comprises two of the lipids or lipid metabolites or derivatives and/or precursors thereof.

[057] In various examples, the culture medium comprises arachidonic acid at a concentration of 100-300 µM and linoleic acid at a concentration of 500-1800 µM.

[058] In various examples, the culture medium comprises prostaglandin E2 at a concentration of 0.0001-0.0009 µM in combination with either linoleic acid at a concentration of 500-1800 µM or arachidonic acid at a concentration of 100-150 µM.

[059] In various examples, the culture medium comprises all three lipids or lipid metabolites or derivatives and/or precursors thereof.

[060] In various examples, the linoleic acid is in a concentration of 500-2000 µM, the arachidonic acid is at a concentration of 100-300 µM, and the prostaglandin E2 is at a concentration of 0.0001-0.0009 µM.

[061] In various examples, the cell culture is a batch, fed-batch, or continuous perfusion cell culture.

[062] In various examples, the mammalian cells are Chinese Hamster Ovary (CHO) cells, Jurkat cells, 293 cells, HeLa cells, CV-1 cells, or 3T3 cells, or a derivative of any of these cells, wherein said CHO cell can be further selected from the group consisting of a CHO-DG44 cell, a CHO-K1 cell, a CHO DXB11 cell, a CHO-S cell, and a CHO GS deficient cell or a derivative of any of these cells.

[063] In various examples, the heterologous protein is a therapeutic protein, an antibody, or a therapeutically effective fragment thereof.

[064] In various examples, the antibody is a monoclonal antibody or fragment thereof.

[065] In various examples, the antibody is a bispecific antibody.

[066] In various examples, the culture medium is a serum-free perfusion medium.

[067] In various examples, the culture medium is optionally (a) chemically defined, (b) hydrolysate-free, or (c) protein-free but optionally includes insulin and/or insulin-like growth factor.

[068] In various examples, the increased productivity is achieved when the total production of the heterologous protein produced by the cell culture is increased by at least 5%, or at least 6%, or at least 7%, or at least 8%, or at least 9%, or at least 10%, or at least 15%, or at least 20%, or at least 25%, or at least 30%, or at least 35%, or at least 40%, or at least 45%, or at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 90%, or at least 100%, or at least 200%, relative the level of total production in a control cell culture that does not include the lipids or lipid metabolites.

[069] In various examples, the increased productivity is achieved when the cell specific productivity (pg/cell/day) of the cell culture is increased by at least 5%, or at least 6%, or at least 7%, or at least 8%, or at least 9%, or at least 10%, or at least 15%, or at least 20%, or at least 25%, or at least 30%, or at least 35%, or at least 40%, or at least 45%, or at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 90%, or at least 100%, or at least 200%, or at least 5-10%, or at least 7.5-20%, or at least 10-30%, or at least 12.5-40%, or at least 15-50%, or at least 5-50% relative the cell specific productivity in a control cell culture that does not include the lipids or lipid metabolites.

[070] In various examples, the growth suppression is sufficient to maintain the cells in a steady state having a viable cell density that is at least 50% lower, at least 40% lower, at least 30% lower, at least 20% lower, at least 15% lower, or at least 10%

lower, or at least 5% lower, or at least 2.5% lower, or 1% or lower relative a control cell culture that does not include the lipids or lipid metabolites.

[071] In various examples, the cell culture on day 2 is changed to a perfusion cell culture.

[072] In various examples, the perfusion rate increases after perfusion has started.

[073] In various examples, the perfusion rate increases from less or equal to 0.5 vessel volumes per day to 5 vessel volumes per day.

[074] In various examples, the perfusion rate increases from less or equal to 0.5 vessel volumes per day to 2 vessel volumes per day.

[075] In various examples, further comprising harvesting the heterologous protein from the cell culture in a continuous manner.

[076] In various examples, the one or more lipids or lipid metabolites or derivatives thereof are added to the cell medium once a cell density of 10 x 10^6 cells/ml to about 120 x 10^6 cells/ml is reached. In one example, the methods and/or growth media disclosed herein comprises 500 µM of linoleic acid, or a derivative thereof, which decreases the VCD by 6-10% relative to cell culture that does not comprise linoleic acid in its growth medium.

[077] In another example, the methods and/or growth media disclosed herein comprises 900 µM of linoleic acid, or a derivative thereof, which decreases the VCD by 11-14% relative to cell culture that does not comprise linoleic acid in its growth medium.

[078] In still another example, the methods and/or growth media disclosed herein comprises 1350 µM of linoleic acid, or a derivative thereof, which decreases the VCD by 11-25% relative to cell culture that does not comprise linoleic acid in its growth medium.

[079] In one example, the methods and/or growth media disclosed herein comprises 1800 µM of linoleic acid, or a derivative thereof, which decreases the VCD by 23-39.8% relative to cell culture that does not comprise linoleic acid in its growth medium.

[080] In another example, the methods and/or growth media disclosed herein comprises 500 µM of arachidonic acid, or a derivative thereof, which decreases the VCD by 15-36% relative to cell culture that does not comprise arachidonic acid in its growth medium.

[081] In another example, the methods and/or growth media disclosed herein comprises 0.001 µM of prostaglandin E2, or a derivative and/or precursor thereof, which decreases the VCD by 17% relative to cell culture that does not comprise arachidonic acid in its growth medium.

[082] In another example, the methods and/or growth media disclosed herein comprises 10 µM of prostaglandin E2, or a derivative and/or precursor thereof, which decreases the VCD by 28% relative to cell culture that does not comprise arachidonic acid in its growth medium.

[083] In another example, the methods and/or growth media disclosed herein comprises 20 µM of prostaglandin E2, or a derivative and/or precursor thereof, which decreases the VCD by 39% relative to cell culture that does not comprise arachidonic acid in its growth medium.

[084] In another example, the methods and/or growth media disclosed herein comprises 60 µM of prostaglandin E2, or a derivative and/or precursor thereof, which decreases the VCD by 30% relative to cell culture that does not comprise arachidonic acid in its growth medium.

[085] In another example, the methods and/or growth media disclosed herein comprises 10 µM of prostaglandin E2, or a derivative and/or precursor thereof, which decreases the VCD by 28% relative to cell culture that does not comprise arachidonic acid in its growth medium.

[086] In one example, the methods and/or growth media disclosed herein comprises 500 µM of linoleic acid, or a derivative thereof, which increases the cell specific productivity by up to 10% relative to cell culture that does not comprise linoleic acid in its growth medium.

[087] In one example, the methods and/or growth media disclosed herein comprises 900 µM of linoleic acid, or a derivative thereof, which increases the cell specific productivity by up to 14% relative to cell culture that does not comprise linoleic acid in its growth medium.

[088] In one example, the methods and/or growth media disclosed herein comprises 1350 µM of linoleic acid, or a derivative thereof, which increases the cell specific productivity by up to 14% relative to cell culture that does not comprise linoleic acid in its growth medium.

[089] In one example, the methods and/or growth media disclosed herein comprises 1800 µM of linoleic acid, or a derivative thereof, which increases the cell specific productivity by up to 30% relative to cell culture that does not comprise linoleic acid in its growth medium.

[090] In one example, the methods and/or growth media disclosed herein comprises 500 µM of arachidonic acid, or a derivative thereof, which increases the cell specific productivity by up to 52% relative to cell culture that does not comprise arachidonic acid in its growth medium.

[091] In one example, the methods and/or growth media disclosed herein comprises 0.001 µM of prostaglandin E2, or a derivative thereof, which increases the cell specific productivity by up to 13.5% relative to cell culture that does not comprise prostaglandin E2 in its growth medium.

[092] In one example, the methods and/or growth media disclosed herein comprises 10 µM of prostaglandin E2, or a derivative thereof, which increases the cell specific productivity by up to 34.4% relative to cell culture that does not comprise prostaglandin E2 in its growth medium.

[093] In one example, the methods and/or growth media disclosed herein comprises 20 µM of prostaglandin E2, or a derivative thereof, which increases the cell specific productivity by up to 31% relative to cell culture that does not comprise prostaglandin E2 in its growth medium.

[094] In one example, the methods and/or growth media disclosed herein comprises 60 µM of prostaglandin E2, or a derivative thereof, which increases the cell specific productivity by up to 31% relative to cell culture that does not comprise prostaglandin E2 in its growth medium.

BRIEF DESCRIPTION OF THE DRAWINGS

[095] **FIG. 1.** The effect of a temperature shift on (A) % percent viability, (B) viable cell density (e^5 cells/mL), and (C) specific productivity (pg/cell/day) of CHO cell line expressing a heterologous protein in perfusion cell culture. Perfusion was started on day 2 of cell culture and gradually was ramped up to an exchange of 2 vessel volumes per day (2VVD). The arrow indicates the day when the temperature was shifted from 37ºC to the indicated value in the legend (29ºC – squares- or 28ºC –triangles-). Further details can be found in Example 1.

[096] **FIG. 2.** Depicts the metabolic pathway of linoleic acid metabolism. High concentrations of exogenous free linoleic acid are shown to diffuse through the cell membrane which is then converted to arachidonic acid. The arachidonic acid is then metabolized through a multi-step reaction to PGE_2, among other downstream metabolites.

[097] **FIG. 3.** Demonstrates that linoleic acid when added to perfusion medium suppresses cell growth and increases cell specific productivity (q_p) in CHO perfusion cell culture. Linoleic acid at various concentrations 500 µM, 900 µM, 1350 µM and 1800 µM was added to the cell culture media and the effects on (A) viable cell density (e^5 cells/ml), (B) percent (%) cell viability and (C) specific productivity (q_p) were determined. Further details can be found in Example 2.

CLAIMS

1. A method of culturing mammalian cells expressing a heterologous protein in a perfusion cell culture, comprising: culturing the mammalian cells in a culture medium comprising an effective amount of one or more lipids or lipid metabolites, wherein the one or more lipids or lipid metabolites comprises 500-2000 µM linoleic acid, 100-600 µM arachidonic acid and/or 0.0001-100 µM prostaglandin E2.

2. The method of claim 1, wherein the culture medium comprises at least two of the lipids or lipid metabolites, preferably
(a) wherein the culture medium comprises arachidonic acid at a concentration of 100-300 µM and linoleic acid at a concentration of 500-1800 µM or
(b) wherein the culture medium comprises prostaglandin E2 at a concentration of 0.0001-0.0009 µM in combination with either linoleic acid at a concentration of 500-1800 µM or arachidonic acid at a concentration of 100-150 µM.

3. The method of one or more of claims 1 to 2, wherein the culture medium comprises three of the lipids or lipid metabolites.

4. The method of one or more of claims 1 to 3, wherein the cell culture on day 2 is changed to a perfusion cell culture, preferably wherein the perfusion rate increases after perfusion has started, preferably wherein the perfusion rate increases from less or equal to 0.5 vessel volumes per day to 5 vessel volumes per day, or from less or equal to 0.5 vessel volumes per day to 2 vessel volumes per day.

5. The method of one or more of claims 1 to 4, wherein the mammalian cells comprise Chinese Hamster Ovary (CHO) cells, Jurkat cells, 293 cells, HeLa cells, CV-1 cells, or 3T3 cells, or a derivative of any of these cells, wherein said CHO cell can be further selected from the group consisting of a CHO-DG44 cell, a CHO-K1 cell, a CHO DXB11 cell, a CHO-S cell, and a CHO GS deficient cell or a mutant thereof.

6. The method of one or more of claims 1 to 5, wherein the heterologous protein is a therapeutic protein, an antibody, or a therapeutically effective fragment thereof, preferably wherein the antibody is a monoclonal antibody or fragment thereof or a bispecific antibody.

7. The method of one or more of claims 1 to 6, wherein the lipid or lipid metabolite or combination thereof results in growth suppression and/or increased productivity, preferably
(a) wherein the total production of the heterologous protein produced by the cell culture is increased by at least 5-50% relative the level of total production in a control cell culture that does not include the lipids or lipid metabolites and/or
(b) wherein the cell specific productivity (pg/cell/day) of the cell culture is increased by at least 5-50% relative the cell specific productivity in a control cell culture that does not include the lipids or lipid metabolites; and/or
(c) wherein cell growth is suppressed at a level which is sufficient to maintain the cells in a steady state having a viable cell density that is at least 5-50% lower relative a control cell culture that does not include the lipids or lipid metabolites.

8. The method of one or more of claims 1 to 7, wherein the culture medium is a serum-free perfusion medium, preferably wherein the culture medium is optionally (a) chemically defined, (b) hydrolysate-free, or (c) protein-free but optionally includes insulin and/or insulin-like growth factor.

9. The method of one or more of claims 1 to 8, further comprising harvesting the heterologous protein from the cell culture.

10. The method of one or more of claims 1 to 9, wherein the one or more lipids or lipid metabolites are added to the cell medium once a cell density of 10×10^6 cells/ml to about 120×10^6 cells/ml is reached.

11. A method of producing a therapeutic protein using the method of any one of the claims 1-10.

12. A method of producing a therapeutic protein from a perfusion cell culture, comprising:

(a) culturing mammalian cells expressing a heterologous protein in a culture medium comprising an effective amount of one or more lipids or lipid metabolites, wherein the one or more lipids or lipid metabolites comprises 500-2000 µM linoleic acid, 100-600 µM arachidonic acid and/or 0.0001-100 µM prostaglandin E2,

(b) harvesting the heterologous protein from the perfusion cell culture.

13. The method of claim 12, wherein

(a) the effective amount of the one or more lipids or lipid metabolites remains constant during cell culture; and/or

(b) wherein the one or more lipids or lipid metabolites are added to the cell medium once a cell density of 10×10^6 cells/ml to about 120×10^6 cells/ml is reached.

14. A perfusion cell culture medium comprising one or more lipids or lipid metabolites comprising 500-2000 µM linoleic acid, 100-600 µM arachidonic acid and/or 0.0001-100 µM prostaglandin E2.

15. Use of the perfusion cell culture medium of claim 14 for culturing mammalian cells in a perfusion culture.

16. Use of the perfusion cell culture medium of claim 14 for suppressing the growth of the perfusion culture and increasing productivity.