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

[026a] In one 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 another aspect of the disclosure, a method of producing a heterologous protein using the method of the disclosure is provided.

[026c] In another aspect of the disclosure, a method of producing a heterologous protein from a perfusion cell culture is provided comprising: (a) culturing mammalian cells expressing the 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.

[026d] In another aspect of the disclosure, a serum-free perfusion cell culture medium is provided comprising one or more lipids or lipid metabolites comprising 500-2000 µM linoleic acid, 300-500 µM arachidonic acid and/or 10-100 µM prostaglandin E2.

[026e] In another aspect of the disclosure, a use of the serum-free perfusion cell culture medium as defined herein for culturing mammalian cells in a perfusion culture is provided.

[026f] In another aspect of the disclosure, a use of the serum-free perfusion cell culture medium as defined herein for suppressing the cell growth of a perfusion culture and increasing cell specific 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

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 claim 1 or 2, wherein the culture medium comprises three of the lipids or lipid metabolites.

4. The method of any one of claims 1 to 3, wherein the culturing of the mammalian cells in the perfusion cell culture starts on day 2, 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 any one 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 any one 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 any one of claims 1 to 6, wherein the lipid or lipid metabolite or combination thereof results in cell growth suppression and/or increased cell specific 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 any one 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 any one of claims 1 to 8, further comprising harvesting the heterologous protein from the cell culture.

10. The method of any one 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 120×10^6 cells/ml is reached.

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

12. The method of claim 11, wherein the heterologous protein is a therapeutic protein.

13. A method of producing a heterologous protein from a perfusion cell culture, comprising:

(a) culturing mammalian cells expressing the 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.

14. The method of claim 13, wherein the heterologous protein is a therapeutic protein.

15. The method of claim 13 or 14, 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 x 10^6 cells/ml to 120 x 10^6 cells/ml is reached.

16. A serum-free perfusion cell culture medium comprising one or more lipids or lipid metabolites comprising 500-2000 µM linoleic acid, 300-500 µM arachidonic acid and/or 10-100 µM prostaglandin E2.

17. Use of the serum-free perfusion cell culture medium of claim 16 for culturing mammalian cells in a perfusion culture.

18. Use of the serum-free perfusion cell culture medium of claim 16 for suppressing the cell growth of a perfusion culture and increasing cell specific productivity.