CLAIMS

1. A method of culturing mammalian cells expressing a heterologous protein in a perfusion cell culture comprising:

   (a) culturing mammalian cells expressing a heterologous protein in a serum-free culture medium;

   (b) culturing the mammalian cells during growth phase by perfusion with a serum-free perfusion medium; and

   (c) maintaining the mammalian cells during production phase by perfusion with a serum-free perfusion medium comprising Fe ions at a concentration of 230 µM to 3.5 mM and a retinoid at a concentration of 10 µM to 400 µM,

   wherein step (b) is optional.

2. A method of reducing cell bleeding in a perfusion cell culture expressing a heterologous protein comprising:

   (a) culturing mammalian cells expressing a heterologous protein in a serum-free culture medium;

   (b) culturing the mammalian cells during growth phase by perfusion with a serum-free perfusion medium; and

   (c) maintaining the mammalian cells during production phase by perfusion with a serum-free perfusion medium comprising Fe ions at a concentration of 230 µM to 3.5 mM and a retinoid at a concentration of 10 µM to 400 µM,

   wherein step (b) is optional.

3. A method of increasing protein production in a perfusion cell culture expressing a heterologous protein comprising:

   (a) culturing mammalian cells expressing a heterologous protein in a serum-free culture medium;

   (b) culturing the mammalian cells during growth phase by perfusion with a serum-free perfusion medium; and

   (c) maintaining the mammalian cells during production phase by perfusion with a serum-free perfusion medium comprising Fe ions at a concentration of 230 µM to 3.5 mM and a retinoid at a concentration of 10 µM to 400 µM,

wherein step (b) is optional.

4. The method of any one of claims 1 to 3, wherein

   (iii) step (c) further comprises maintaining a cell density by cell bleeding, and/or

   (iv) prior to step (c) the retinoid concentration in the mammalian cell culture is adjusted to the retinoid concentration in the serum-free perfusion medium of step (c), preferably by bolus addition.

5. The method of any one of the preceding claims, wherein the retinoid is retinol, retinal, retinoic acid or a retinyl ester, preferably retinol or a retinyl ester, preferably retinyl acetate or retinyl palmitate, more preferred retinyl acetate.

6. The method of any one of the preceding claims, wherein the Fe ion is provided as $FeSO_4$, Fe-citrate, Fe-transferrin, iron choline citrate, Fe-EDTA, $Fe(NO_3)_3$, $FeCl_2$, $FeCl_3$, or combinations thereof, preferably $FeSO_4$, Fe-citrate, $Fe(NO_3)_3$, Fe-transferrin, or combinations thereof, more preferred Fe-citrate.

7. The method of any one of the preceding claims, wherein the mammalian cell is a Chinese hamster ovary (CHO) cell, preferably a CHO-DG44 cell, a CHO-K1 cell, a CHO DXB11 cell, a CHO-S cell, a CHO GS deficient cell or a derivative of any of these cells.

8. The method of any one of the preceding claims, further comprising harvesting the heterologous protein from the perfusion cell culture.

9. The method of any one of the preceding claims, wherein the heterologous protein is a therapeutic protein, preferably selected from the group consisting of an antibody, a fusion protein, a cytokine and a growth factor.

10. The method of any one of the preceding claims, wherein the serum-free perfusion medium is chemically defined.

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

12. A serum-free perfusion medium comprising Fe ions at a concentration ranging from 230 µM to 3.5 mM and a retinoid in a concentration ranging from 10 µM to 400 µM.

13. Use of the serum-free perfusion medium of claim 12 for culturing mammalian cells in a perfusion culture during production phase.

14. Use of the serum-free perfusion medium of claim 12 for reducing the total cell bleed volume in a perfusion culture.

15. Use of the serum-free perfusion medium of claim 12 for increasing protein production in a perfusion cell culture.