CLAIMS

1. A method of increasing cell density, viability, and/or titer in a cell culture medium comprising steps of:

(a) providing cells in a cell culture medium to start a cell culture process; and

(b) adding a composition comprising iron to said cell culture medium during the cell culture process such that the concentration of iron in the cell culture medium is increased over the course of the cell culture process.

2. The method of claim 1, wherein the composition comprising iron is selected from the group consisting of $FeSO_4$, Fe-citrate, Fe-transferrin, Fe-chloride, Fe-nitrate, Fe-EDTA, $Fe(NO_3)_3$, $FeCl_2$, $FeCl_3$ and combinations thereof.

3. The method of claim 1, wherein the composition comprising iron is added after day 0 of the cell culture process.

4. The method of claim 1, wherein the composition comprising iron is added on or after day 1 of the cell culture process.

5. The method of claim 1, wherein the composition comprising iron is added on or after day 3 of the cell culture process.

6. The method of claim 1, wherein the composition comprising iron is added on or after day 6 of the cell culture process.

7. The method of claim 1, wherein the composition comprising iron is added at multiple time points during the cell culture process.

8. The method of claim 1, wherein the concentration of iron in the cell culture medium after addition of the composition comprising iron ranges between 100 μM and 5 mM.

9. The method of claim 1, wherein the concentration of iron in the cell culture medium after addition of the composition comprising iron ranges between 300 μM and 1 mM.

10. The method of claim 1, wherein the concentration of iron in the cell culture medium after addition of the composition comprising iron is 1mM.

11. The method of claim 1, wherein the cells are mammalian cells.

12. The method of claim 11, wherein the mammalian cells are selected from BALB/c mouse myeloma line, human retinoblasts (PER.C6), monkey kidney cells, human embryonic kidney line (293), baby hamster kidney cells (BHK), Chinese hamster ovary cells (CHO), mouse sertoli cells, African green monkey kidney cells (VERO-76), human cervical carcinoma cells (HeLa), canine kidney cells, buffalo rat liver cells, human lung cells, human liver cells, mouse mammary tumor cells, TRI cells, MRC 5 cells, FS4 cells, or human hepatoma line (Hep G2).

13. The method of claim 12, wherein the mammalian cells are CHO cells.

14. The method of claim 1, wherein the cell culture process is a fed batch culture process.

15. The method of claim 1, wherein the cell culture process is a batch-refeed culture process.

16. The method of claim 1, wherein the cell culture process is a perfusion culture process.

17. The method of claim 1, wherein the cell culture process is a large-scale production culture process.

18. The method of claim 17, wherein the volume of the cell culture medium is at least about 500 L.

19. The method of claim 1, wherein the cell culture medium does not contain hydrolysates.

20. The method of claim 1, wherein the cell culture medium contains hydrolysates.

21. The method of claim 1, wherein the cells carry a gene that encodes a recombinant protein.

22. The method of claim 21, wherein the recombinant protein is an antibody or fragment thereof.

23. The method of claim 22, wherein the antibody is an anti-IL-22 antibody.

24. The method of claim 22, wherein the antibody is an anti-GDF-8 antibody.

25. The method of claim 22, wherein the antibody is Myo29.

26. The method of claim 22, wherein the antibody is a single domain antibody.

27. The method of claim 26, wherein the single domain antibody is an anti-TNF nanobody.

28. The method of claim 21, wherein the recombinant protein is a glycoprotein.

29. The method of claim 21, wherein the recombinant protein is a therapeutic protein.

30. The method of claim 29, wherein the therapeutic protein is a growth factor, a clotting factor, a cytokine, a fusion protein, a pharmaceutical drug substance, a vaccine, an enzyme, or a Small Modular ImmunoPharmaceutical™ (SMIP).

31. The method of any one of claims 21-30, wherein the method further comprises purifying the recombinant protein.

32. A recombinant protein produced from cells cultured according to the method of any one of claims 21-31.

33. A method of preventing or delaying cell death in a cell culture, the method comprising a step of adding a composition comprising iron at one or more time points subsequent to the beginning of the cell culture process.

34. A method of inhibiting apoptosis in a cell culture, the method comprising a step of adding a composition comprising iron at one or more time points subsequent to the beginning of the cell culture process.

35. The method of claim 33 or 34, wherein the composition comprising iron is added after day 0.

36. The method of claim 33 or 34, wherein the composition comprising iron is added after day 3.

37. The method of claim 33 or 34, wherein the composition comprising iron is added on day 6.

38. The method of claim 33 or 34, wherein the iron is added in an amount to effect a concentration in the cell culture medium ranging between 100 μM and 5 mM.

39. The method of claim 33 or 34, wherein the iron is added in an amount to effect a concentration in the cell culture medium ranging between 300 μM and 1 mM.

40. The method of claim 33 or 34, wherein the iron is added in an amount to effect a concentration in the cell culture medium of 1 mM.

41. The method of claim 33 or 34, wherein the cell culture process is a fed batch culture process.

42. The method of claim 33 or 34, wherein the cell culture is a batch-refeed culture.

43. The method of claim 33 or 34, wherein the cell culture is a perfusion culture.

44. The method of claim 33 or 34, wherein the cell culture is a large-scale production culture.

45. The method of claim 44, wherein the volume of the cell culture is at least about 500 L.

46. The method of claim 33 or 34, wherein the composition comprising iron is selected from the group consisting of $FeSO_4$, Fe-citrate, Fe-transferrin, Fe-chloride, Fe-nitrate, Fe-EDTA, $Fe(NO_3)_3$, $FeCl_2$, $FeCl_3$ and combinations thereof.

47. A kit for making a cell culture medium comprising:

(a) one or more reagents for making an initial cell culture medium; and

(b) a separate composition comprising iron;

(c) an instruction to add the separate composition comprising iron to the initial cell culture medium at one or more time points during the course of a cell culture process to increase cell density, viability, and/or titer.

48. The kit of claim 47, wherein the separate composition is in an amount to effect an iron concentration ranging between 100 µM and 5 mM in the initial cell culture medium.

49. The kit of claim 47, wherein the separate composition is in an amount to effect an iron concentration ranging between 100 µM and 5 mM in a cell culture of least 500L.

50. The kit of claim 47, wherein the separate composition is selected from the group consisting of $FeSO_4$, Fe-citrate, Fe-transferrin, Fe-chloride, Fe-nitrate, Fe-EDTA, $Fe(NO_3)_3$, $FeCl_2$, $FeCl_3$ and combinations thereof.

51. The kit of claim 47, wherein the kit further comprises supplementary components for a fed batch culture.

52. The kit of claim 47, wherein the kit further comprises supplementary components for a batch-refeed culture.

53. The kit of claim 47, wherein the kit further comprises supplementary components for a perfusion culture.

54. The kit of any one of claims 51-53, wherein the supplementary components are selected from the group consisting of hormones and/or other growth factors, inorganic ions, buffers, vitamins, nucleosides or nucleotides, trace elements, amino

acids, lipids, glucose or other energy sources, and combinations thereof.

CLAIMS

1. A method of increasing cell density, viability, and/or titer in a cell culture medium comprising steps of:

(a) providing cells in a cell culture medium to start a cell culture process; and

(b) adding a composition comprising iron to said cell culture medium on or after day 3 or on day 3 and after day 3 of the cell culture process such that the concentration of iron in the cell culture medium is increased over the course of the cell culture process.

2. The method of claim 1, wherein the composition comprising iron is selected from the group consisting of $FeSO_4$, Fe-citrate, Fe-transferrin, Fe-chloride, Fe-nitrate, Fe-EDTA, $Fe(NO_3)_3$, $FeCl_2$, $FeCl_3$ and combinations thereof.

3. The method of claim 1, wherein the composition comprising iron is added on or after day 6 of the cell culture process.

4. The method of claim 1, wherein the composition comprising iron is added at multiple time points during the cell culture process.

5. The method of claim 1, wherein the concentration of iron in the cell culture medium after addition of the composition comprising iron ranges between 100 μM and 5 mM.

6. The method of claim 1, wherein the concentration of iron in the cell culture medium after addition of the composition comprising iron ranges between 300 μM and 1 mM.

7. The method of claim 1, wherein the concentration of iron in the cell culture medium after addition of the composition comprising iron is 1mM.

8. The method of claim 1, wherein the cells are mammalian cells.

9. The method of claim 8, wherein the mammalian cells are selected from BALB/c mouse myeloma line, human retinoblasts (PER.C6), monkey kidney cells, human embryonic kidney line (293), baby hamster kidney cells (BHK), Chinese hamster ovary cells (CHO), mouse sertoli cells, African green monkey kidney cells (VERO-76), human cervical carcinoma cells (HeLa), canine kidney cells, buffalo rat liver cells, human lung cells, human liver cells, mouse mammary tumor cells, TRI cells, MRC 5 cells, FS4 cells, or human hepatoma line (Hep G2).

10. The method of claim 9, wherein the mammalian cells are CHO cells.

11. The method of claim 1, wherein the cell culture process is a fed batch culture process.

12. The method of claim 1, wherein the cell culture process is a batch-refeed culture process.

13. The method of claim 1, wherein the cell culture process is a perfusion culture process.

14. The method of claim 1, wherein the cell culture process is a large-scale production culture process.

15. The method of claim 14, wherein the volume of the cell culture medium is at least about 500 L.

16. The method of claim 1, wherein the cell culture medium does not contain hydrolysates.

17. The method of claim 1, wherein the cell culture medium contains hydrolysates.

18. The method of claim 1, wherein the cells carry a gene that encodes a recombinant protein.

19. The method of claim 18, wherein the recombinant protein is an antibody or fragment thereof.

20. The method of claim 19, wherein the antibody is an anti-IL-22 antibody.

21. The method of claim 19, wherein the antibody is an anti-GDF-8 antibody.

22. The method of claim 19, wherein the antibody is Myo29.

23. The method of claim 19, wherein the antibody is a single domain antibody.

24. The method of claim 23, wherein the single domain antibody is an anti-TNF nanobody.

25. The method of claim 18, wherein the recombinant protein is a glycoprotein.

26. The method of claim 18, wherein the recombinant protein is a therapeutic protein.

27. The method of claim 26, wherein the therapeutic protein is a growth factor, a clotting factor, a cytokine, a fusion protein, a pharmaceutical drug substance, a vaccine, an enzyme, or a Small Modular ImmunoPharmaceutical™ (SMIP).

28. The method of any one of claims 18-27, wherein the method further comprises purifying the recombinant protein.

29. A method of preventing or delaying cell death in a cell culture, the method comprising a step of adding a composition comprising iron at one or more time

points on or after day 3 or on day 3 and after day 3 of the cell culture process.

30. A method of inhibiting apoptosis in a cell culture, the method comprising a step of adding a composition comprising iron at one or more time points subsequent to the beginning of the cell culture process.

31. The method of claim 29 or 30, wherein the composition comprising iron is added on day 6.

32. The method of claim 29 or 30, wherein the iron is added in an amount to effect a concentration in the cell culture medium ranging between 100 μM and 5 mM.

33. The method of claim 29 or 30, wherein the iron is added in an amount to effect a concentration in the cell culture medium ranging between 300 μM and 1 mM.

34. The method of claim 29 or 30, wherein the iron is added in an amount to effect a concentration in the cell culture medium of 1 mM.

35. The method of claim 29 or 30, wherein the cell culture process is a fed batch culture process.

36. The method of claim 29 or 30, wherein the cell culture is a batch-refeed culture.

37. The method of claim 29 or 30, wherein the cell culture is a perfusion culture.

38. The method of claim 29 or 30, wherein the cell culture is a large-scale production culture.

39. The method of claim 38, wherein the volume of the cell culture is at least about 500 L.

40. The method of claim 29 or 30, wherein the composition comprising iron is selected from the group consisting of $FeSO_4$, Fe-citrate, Fe-transferrin, Fe-chloride, Fe-nitrate, Fe-EDTA, $Fe(NO_3)_3$, $FeCl_2$, $FeCl_3$ and combinations thereof.