CHO cell in culture and/or for introducing at least one macromolecule into at least one CHO cell in culture.

[0017] The present disclosure also provides a composition comprising the CHO cell high density growth medium of the present disclosure and at least one component selected from the group consisting of at least one CHO cell, one or more agents for the introduction of at least one macromolecule into at least one cell (i.e., one or more transfection agents, which may preferably be one or more cationic lipids), one or more expression enhancer compositions, one or more growth modulator compositions, and one or more macromolecules comprising an expressible nucleic acid comprising at least one protein coding region in addition to one or more elements enabling the expression thereof in a host cell under appropriate conditions that allow for protein expression.

[0018] In one aspect, there is provided a method for producing a recombinant protein in cultured suspension CHO cells, said method comprising: obtaining a suspension culture of CHO cells, said CHO cells being adapted for growth under high density culture conditions, and culturing said CHO cells in suspension in a culture medium at a cell density of between $2x10^6$ to $2x10^7$ cells/ml, transfecting said CHO cells in said culture medium with an expression vector in the presence of a transfection reagent, the expression vector comprising a nucleic acid sequence capable of producing an expressed protein; incubating said transfected CHO cells for a first period of time of up to 2 days, contacting said transfected CHO cells with at least one expression enhancer composition and at least one growth modulator composition, wherein said expression enhancer composition comprises at least one of valproic acid, sodium propionate, sodium butyrate, lithium acetate, dimethyl sulfoxide (DMSO), galactose, amino acids, or any combinations of the aforementioned, and wherein said growth modulator composition comprises a plurality of amino acids, each amino acids having a concentration in the range of 0.1 mg/ml to 8 mg/ml, and wherein said growth modulator composition has an osmolality of 1000 mOsm/kg to 1500 mOsm/kg, incubating said transfected CHO cells in the presence of said expression enhancer and said growth modulator for a second period of time under conditions such that said expression vector expresses said protein, wherein the second period of time is up to 20 days; and harvesting said transfected CHO cells and isolating the expressed protein, wherein said method does not require replacement, replenishment, or supplementation of the culture medium following the transfection step.

[0019] In some examples, a method for producing a recombinant protein in cultured suspension CHO cells may include, after the aforementioned second period of time, contacting the transfected cells a second time with said growth modulator composition and incubating said transfected cells for at least a third period of time prior to harvesting the transfected CHO cells and isolating the expressed protein.

[0020] In some examples, the aforementioned third period of time may be up to about 20 days, up to about 15 days, up to about 14 days, up to about 13 days, up to about 12 days,

WHAT IS CLAIMED IS:

1. A method for producing a recombinant protein in cultured suspension CHO cells, said method comprising:

obtaining a suspension culture of CHO cells, said CHO cells being adapted for growth under high density culture conditions,

culturing said CHO cells in suspension in a culture medium at a cell density of between $2x10^6$ to $2x10^7$ cells/ml;

transfecting said CHO cells in said culture mediumwith an expression vector in the presence of a transfection reagent, said expression vector comprising a nucleic acid sequence capable of producing an expressed protein;

incubating said transfected CHO cells for a first period of time of up to 2 days;

contacting said transfected CHO cells with at least one expression enhancer composition and at least one growth modulator composition, wherein said expression enhancer composition comprises at least one of valproic acid, sodium propionate, sodium butyrate, lithium acetate, dimethyl sulfoxide (DMSO), galactose, amino acids, or any combinations of the aforementioned, and wherein said growth modulator composition comprises a plurality of amino acids, each amino acids having a concentration in the range of 0.1 mg/ml to 8 mg/ml, and wherein said growth modulator composition has an osmolality of 1000 mOsm/kg to 1500 mOsm/kg;

incubating said transfected CHO cells in the presence of said expression enhancer and said growth modulator for a second period of time under conditions such that said expression vector expresses said protein, wherein the second period of time is up to 20 days; and

harvesting said transfected CHO cells and isolating the expressed protein, wherein said method does not require replacement, replenishment, or supplementation of the culture medium following the transfection step.

2. The method according to claim 1, wherein after said second period of time, contacting said transfected cells a second time with said growth modulator composition and incubating said transfected cells for a third period of time prior to harvesting said transfected CHO cells and isolating the expressed protein.

3. The method according to claim 2, wherein said third period of time is up to 20 days.

4. The method according to claim 1, wherein after said transfected cells are contacted with the expression enhancer composition and the growth modulator composition, said transfected cells are cultured at a temperature of less than 37ºC and greater than 30ºC.

5. The method according to claim 4, wherein said transfected cells are cultured at a temperature of less than 35ºC and greater than 31ºC.

6. The method according to claim 4, wherein said transfected cells are cultured at a temperature of 32ºC.

7. The method according to claim 1, wherein said suspension CHO cells are CHO-S cells, or a derivative of CHO-S cells adapted for growth at greater than $2x10^6$ cells/ml.

8. The method according to claim 1, wherein said suspension CHO cells are CHO-S-2H2 cells or CHO-S-clone 14 cells.

9. The method according to claim 1, wherein said suspension CHO cells are cultured prior to transfection at a cell density of between $3x10^6$ to $15x10^6$ cells/ml.

10. The method according to claim 1, wherein said transfection reagent comprises a cationic lipid or a polymeric amine-based transfection reagent.

11. The method according to claim 1, wherein said transfection reagent comprises polyethylenimine (PEI) or a chemical derivative thereof.

12. The method according to claim 11, wherein the PEI is linear PEI.

13. The method according to claim 1, wherein the transfection reagent comprises a cationic lipid.

14. The method of claim 13, wherein cationic lipid is contacted with said expression vector to form a transfection complex prior to transfecting said suspension CHO cells.

15. The method of claim 14, wherein said transfection complex is formed at a temperature of greater than 0°C and less than 20°C, less than 15°C, less than 10°C, less than 8°C, or less than 5°C.

16. The method according to claim 1, wherein the volume of the suspension culture prior to transfection is in the range of 20 mL to 1500 ml.

17. The method according to claim 1, wherein the volume of the suspension culture prior to transfection is 1000 ml.

18. The method according to claim 1, wherein said expression enhancer composition comprises at least two or more of valproic acid, sodium propionate, sodium butyrate, lithium acetate, dimethyl sulfoxide (DMSO), galactose, amino acids, or any combinations of the aforementioned.

19. The method according to claim 1, wherein said expression enhancer composition comprises at least three or more of valproic acid, sodium propionate, sodium butyrate, lithium acetate, dimethyl sulfoxide (DMSO), galactose, amino acids, or any combinations of the aforementioned.

20. The method according to claim 1, wherein said expression enhancer composition comprises valproic acid, sodium propionate, and sodium butyrate.

21. The method according to claim 1, wherein said expression enhancer composition further comprises a composition that reduces clumping of suspension cells by at least 85%, at least 80%, at least 75%, at least 70%, at least, 65%, at least 60%, at least 55% or at least 50%, wherein said composition that reduces clumping of suspension cells comprises dextran sulfate.

22. The method according to claim 21, wherein the concentration of dextran sulfate in said expression enhancer composition is between 10 mg/ml to 200 mg/ml.

23. The method according to claim 1, wherein the expression enhancer composition comprises valproic acid.

24. The method according to claim 23, wherein the concentration of valproic acid in the expression enhancer composition is in the range of 5 mg/ml to 50 mg/ml.

25. The method according to claim 23, wherein the concentration of valproic acid in the expression enhancer composition is in the range of 20 mM to 150 mM.

26. The method according to claim 23, wherein the concentration of valproic acid in the expression enhancer composition is 50 mM to 150 mM.

27. The method according to claim 23, wherein the concentration of valproic acid in the expression enhancer composition is 10 mg/ml to 20 mg/ml.

28. The method according to claim 1, wherein the expression enhancer composition comprises sodium propionate.

29. The method according to claim 28, wherein the final concentration of sodium propionate in the culture is in the range of 0.2 mM to 100 mM.

30. The method according to claim 28, wherein the final concentration of sodium propionate in the culture is in the range of 0.1 mg/ml to 0.2 mg/ml.

31. The method according to claim 28, wherein the final concentration of sodium propionate in the expression enhancer composition is 0.5 mM to 5 mM.

32. The method according to claim 1, wherein the expression composition comprises sodium butyrate.

33. The method according to claim 32, wherein the final concentration of sodium butyrate in the culture is in the range of 0.01 mM to 1 mM, 5 mg/L to 20 mg/L.

34. The method according to claim 1,wherein the volume of the suspension culture is in the range of 25 mL to 50 L.

35. The method according to claim 1,wherein the volume of the suspension culture is in the range of 100 mL to 1 L.

36. The method according to claim 1,wherein the volume of the suspension culture is in the range of 200 mL to 500 ml.

37. The method according to claim 1, wherein the cell density of the transfection step is in the range of $2x10^6$ to $6x10^6$ cells/ml.

38. The method according to claim 1, wherein said culture medium is a serum-free/protein-free chemically defined culture medium that promotes the growth of transfected CHO cells at densities in excess of 2 x 10^6 cells/ml to 2 x 10^7 cells/ml with cell viability remaining in excess of 75%, 80%, 85%, 90%, 95% after the transfection thereof with said expression vector.

39. The method according to claim 1, further comprising purifying said expressed protein.

40. The method according to claim 1, wherein said CHO cells express one or more expression enhancing proteins selected from the group consisting of AKT, P18, P21, Bcl-X_L, and PKBa.

41. The method according to claim 40, wherein said CHO cells transiently express one or more of said expression enhancing proteins.

42. The method according to claim 40, wherein said CHO cells stably express one or more of said expression enhancing proteins.

43. The method according to claim 1, wherein said first period of time is in the range of 12 hrs to 2 days.

44. The method according to claim 1, wherein said first period of time is about 22 hrs.

45. The method according to claim 1, wherein said growth modulator composition further comprises glucose.

46. The method according to claim 45, wherein the osmolality of said glucose in said growth modulator composition is between 500 mOsm/kg to 700 mOsm/kg.

47. The method according to claim 45, wherein the concentration of said glucose is in the range of 85 mg/ml to 115 mg/ml.

48. The method according to claim 1, wherein the volume of the culture when the transfected CHO cells are harvested is less than about 150% of the volume of the culture at the time the suspension cells were transfected, wherein the increase in volume is not due to the addition, supplementation, or replacement of culture medium.

49. The method according to claim 1, wherein the volume of the culture when the transfected CHO cells are harvested is less than 150% the volume of the culture at the time the suspension cells were transfected, wherein the increase in volume is due to the addition of the growth enhancer and the expression enhancer compositions.