(Chauhan *et al.*, J Exp Med. 2008 Sep 1;205(9):2065-74), and most recently, severe plasmodium falciparum malaria (Larkin *et al.*, PLoS Pathog. 2009 Mar;5(3):e1000349).

[0018] The ADAMTS13 protease is a 190 kDa glycosylated protein produced predominantly by the liver (Levy *et al.*, Nature. 2001; 413:488-494; Fujikawa *et al.*, Blood. 2001; 98:1662-1666; Zheng *et al.*, J Biol Chem. 2001; 276:41059-41063; Soejima *et al.*, J Biochem (Tokyo). 2001; 130:475-480; and Gerritsen *et al.*, Blood. 2001; 98:1654-1661). Much like the higher order rVWF multimers, recombinant expression of the large ADAMTS13 in mammalian cell culture presents many challenges.

[0019] Therefore, there is a need to provide cell culture conditions, particularly large-scale manufacturing culture conditions, that provide consistent total protein yield and/or consistent total and specific activity of the proteins produced between different cell cultures. Consistency among cultures in large-scale manufacturing processes is of importance in the manufacture of therapeutic proteins. There is also a need for cell culture conditions for large-scale production of rVWF with a multimeric distribution and specific Ristocetin activity comparable or higher than VWF as it is present in normal human plasma. Similarly, as ADAMTS proteins have been implicated in a number of diseases and conditions, there is a need in the art for methods of large scale production of recombinant ADAMTS proteins having high specific activities, which are suitable for pharmaceutical formulation and administration. The present invention satisfies these and other needs in the art for the production of recombinant Von Willebrand Factor and recombinant ADAMTS13.

SUMMARY

[0020] In certain examples, the present disclosure describes the surprising finding that supplementation of cell culture media used to express recombinant Von Willebrand Factor (rVWF) and recombinant ADAMTS13 (rA13) results in significantly improved protein expression and enzymatic activity. In an aspect, the present disclosure refers to a composition comprising recombinant Von Willebrand Factor (rVWF), wherein the composition contains at least 20% of the rVWF is present in a high molecular weight VWF multimer of more than 10 dimers, wherein the composition is purified from a cell culture supernatant ~~is~~ recovered from cells

cultured in a medium, wherein the cell culture supernatant has ~~supplemented to provide~~ a final copper concentration between 2.4 µg/L and 20 µg/L, and wherein the $NH4^+$ content of the cell culture supernatant is ~~maintained~~ at a concentration below 4 mM, ~~wherein the cell density is maintained at less than $2.5x10^6$ cells per mL during the step of cell culturing,~~ and wherein the supernatant has a rVWF specific ristocetin cofactor activity of at least 30 mU/µg rVWF.

[0021] In an example, the present disclosure herein describes a method for producing a recombinant Von Willebrand Factor (rVWF) composition, the method comprising the steps of: (a) providing a basal cell culture media; (b) supplementing the basal cell culture media with copper to provide a

CLAIMS

1. A composition comprising recombinant Von Willebrand Factor (rVWF), wherein the composition contains at least 0.5 IU ristocetin cofactor activity per mL, wherein at least 20% of the rVWF is present in a high molecular weight VWF multimer of more than 10 dimers, wherein the composition is purified from a cell culture supernatant ~~is~~ recovered from cells cultured in a medium, wherein the cell culture supernatant has ~~supplemented to provide~~ a final copper concentration between 2.4 µg/L and 20 µg/L, and wherein the NH_4^+ content of the cell culture supernatant is ~~maintained~~ at a concentration below 4 mM~~, wherein the cell density is maintained at less than 2.5×10^6 cells per mL during the step of cell culturing~~, and wherein the supernatant has a rVWF specific ristocetin cofactor activity of at least 30 mU/µg rVWF.

2. The composition of claim 1, wherein the cell culture media ~~is supplemented with~~comprises a hydrolysate ~~prior to culturing the one or more cells~~.

3. The composition of claim 2, wherein the hydrolysate is a plant hydrolysate.

4. The composition of claim 3, wherein the hydrolysate is a soy hydrolysate.

5. The composition according to any one of claims 1 to 4, wherein the cell culture media is an animal protein free culture media.

6. The composition according to any one of claims 1 to 4, wherein the cell culture media is a protein free culture media.

7. The composition according to any one of claims 1 ~~to 6~~, wherein the cell culture media is a chemically defined culture media.

~~8. The composition according to any one of claims 1 to 7, wherein the final copper concentration of the copper supplemented basal cell culture media is at least 4 µg/L copper.~~

~~9. The composition according to any one of claims 1 to 8, wherein the final copper concentration of the copper-supplemented basal cell culture media is between 2.4 µg/L and 20 µg/L copper.~~

~~10.~~8. The composition according to any one of claims 1 to ~~9~~7, wherein the copper supplementing the cell culture media is provided as a copper salt, a copper chelate, or a combination thereof.

~~11.~~9. The composition of claim ~~10~~8, wherein the copper salt is selected from the group consisting of copper sulfate, copper acetate, copper carbonate, copper chloride, copper hydroxide, copper nitrate, and copper oxide.

~~12.~~10. The composition according to any one of claims 1 to ~~11~~9, wherein the ~~one or more~~ cells are mammalian cells.

~~13.~~11. The composition of claim ~~12~~10, wherein the mammalian cells are CHO cells.

~~14. The composition according to any one of claims 1 to 13, wherein culturing the one or more cells comprises batch cultivation of the cells.~~

~~15. The composition according to any one of claims 1 to 13, wherein culturing the one or more cells comprises continuous cultivation of the cells.~~

~~16. The composition of claim 15, wherein the continuous cultivation of cells is performed in chemostatic mode.~~

~~17. The composition of claim 15, wherein the continuous cultivation of cells is performed in perfusion mode.~~

~~18. The composition according to any one of claims 1 to 17, wherein the one or more cells is cultured in at least 100 L of the supplemented cell culture media.~~

~~19. The composition according to any one of claims 1 to 18, wherein the cell density is maintained at less than $2.5x10^6$ cells per mL during the step of culturing the one or more cells.~~

~~20. The composition of claim 19, wherein the cell density is maintained at less than $2.0x10^6$ cells per mL during the step of culturing the one or more cells.~~

~~21. The composition of claim 19, wherein the cell density is maintained at less than $1.5x10^6$ cells per mL during the step of culturing the one or more cells.~~

~~22. The composition according to any one of claims 1 to 21, wherein the step of recovering at least a portion of the culture supernatant comprises filtration or centrifugation to remove cells from the portion of culture supernatant.~~

~~23.~~12. The composition according to any one of claims 1 to ~~22~~11, wherein the recovered supernatant has a rVWF specific ristocetin cofactor activity of at least 40 mU/µg rVWF.

~~24.~~13. The composition of claim ~~23~~12, wherein the recovered supernatant has a rVWF specific ristocetin cofactor activity of at least 50 mU/µg rVWF.

~~25.~~14. The composition of claim ~~23~~12, wherein the recovered supernatant has a rVWF specific ristocetin cofactor activity of at least 60 mU/µg rVWF.

~~26.~~15. The composition of claim ~~23~~12, wherein the recovered supernatant has a rVWF specific ristocetin cofactor activity of at least 70 mU/µg rVWF.

~~27.~~16. The composition of claim ~~23~~12, wherein the recovered supernatant has a rVWF specific ristocetin cofactor activity of at least 80 mU/µg rVWF.

~~28.~~17. The composition according to any one of claims 1 to ~~27~~16, wherein at least 10% of the rVWF in the supernatant is present in a high molecular weight VWF multimer of more than 10 dimers.

~~29.~~18. The composition according to any one of claims 1 to ~~27~~16, wherein at least 20% of the rVWF in the supernatant is present in a high molecular weight VWF multimer of more than 10 dimers.

~~30.~~19. The composition according to any one of claims 1 to ~~27~~16, wherein at least 30% of the rVWF in the supernatant is present in a high molecular weight VWF multimer of more than 10 dimers.

~~31.~~20. The composition according to any one of claims 1 to ~~30~~19, wherein the supernatant contains high molecular weight VWF multimers of 14 to 22 dimers.

~~32.~~21. The composition according to any one of claims 1 to 31, wherein the composition further comprises recombinant Factor VIII (rFVIII).

~~33.~~22. The composition of claim 32, wherein the ratio of rVWF to rFVIII is at least 10:1.

~~34.~~23. The composition of claim 32 or 33, wherein the composition is formulated for pharmaceutical administration.

~~35.~~24. The composition according to any one of claims 32 to 34, wherein the composition is formulated for intravenous administration.

~~36. The composition according to any one of claims 1 to 35, wherein rVWF is co-expressed with rFVIII.~~

~~37. The composition of claim 36, wherein rVWF is further purified away from at least 50% of the rFVIII present in the recovered supernatant.~~

~~38. The composition of claim 37, wherein the ratio of rVWF to rFVIII after the purification step is at least 10:1.~~

~~39.~~25. The composition according to any one of claims 1 to ~~38~~20, wherein rVWF is further enriched.

~~40. A cell culture supernatant comprising recombinant Von Willebrand Factor (rVWF), wherein at least 20% of the rVWF is present in a high molecular weight VWF~~

multimer of more than 10 dimers wherein the cell culture supernatant has been supplemented to provide a final copper concentration of at least 2.4 µg/L, and wherein the NH_4^+ content of the cell culture supernatant is maintained at a concentration below 4 mM or below 10 mM.

41. The cell culture supernatant of claim 40, wherein the cell culture supernatant is recovered from cells cultured in a copper supplemented cell culture media, wherein one or more cells comprise a nucleic acid encoding a rVWF protein such that rVWF is expressed and excreted from the cells into the cell culture supernatant.

42. The cell culture supernatant of claim 40 or 41, wherein the cell culture media is supplemented with a hydrolysate prior to culturing the one or more cells.

43. The cell culture supernatant of claim 42, wherein the hydrolysate is a plant hydrolysate.

44. The cell culture supernatant of claim 43, wherein the hydrolysate is a soy hydrolysate.

45. The cell culture supernatant according to any one of claims 40 to 44, wherein the cell culture media is an animal protein free culture media

46. The cell culture supernatant according to any one of claims 40 to 44, wherein the cell culture media is a protein free culture media.

47. The cell culture supernatant according to any one of claims 40 to 46, wherein the cell culture media is a chemically defined culture media.

48. The cell culture supernatant according to any one of claims 40 to 47, wherein the final copper concentration of the copper supplemented cell culture media is at least 4 µg/L copper.

49. The cell culture supernatant according to any one of claims 40 to 48, wherein the final copper concentration of the copper supplemented cell culture media is between 2.4 µg/L and 20 µg/L copper.

50. The cell culture supernatant according to any one of claims 40 to 49, wherein the copper supplementing the cell culture media is provided as a copper salt, a copper chelate, or a combination thereof.

51. The cell culture supernatant of claim 50, wherein the copper salt is selected from the group consisting of copper sulfate, copper acetate, copper carbonate, copper chloride, copper hydroxide, copper nitrate, and copper oxide.

52. The cell culture supernatant according to any one of claims 40 to 51, wherein the one or more cells are mammalian cells.

53. The cell culture supernatant of claim 52, wherein the mammalian cells are CHO cells.

54. The cell culture supernatant according to any one of claims 40 to 53, wherein culturing the one or more cells comprises batch cultivation of the cells.

55. The cell culture supernatant according to any one of claims 40 to 53, wherein culturing the one or more cells comprises continuous cultivation of the cells.

56. The cell culture supernatant of claim 55, wherein the continuous cultivation of cells is performed in chemostatic mode.

57. The cell culture supernatant of claim 55, wherein the continuous cultivation of cells is performed in perfusion mode.

58. The cell culture supernatant according to any one of claims 40 to 57, wherein the one or more cells is cultured in at least 100 L of the supplemented cell culture media.

59. The cell culture supernatant according to any one of claims 40 to 58, wherein the cell density is maintained at less than 2.5×10^6 cells per mL during the step of culturing the one or more cells.

60. The cell culture supernatant of claim 59, wherein the cell density is maintained at less than 2.0×10^6 cells per mL during the step of culturing the one or more cells.

61. The cell culture supernatant of claim 59, wherein the cell density is maintained at less than $1.5x10^6$ cells per mL during the step of culturing the one or more cells.

62. The cell culture supernatant according to any one of claims 40 to 61, wherein the step of recovering at least a portion of the culture supernatant comprises filtration or centrifugation to remove cells from the portion of culture supernatant.

63. The cell culture supernatant according to any one of claims 40 to 62, wherein the recovered supernatant has a rVWF specific ristocetin cofactor activity of at least 40 mU/µg rVWF.

64. The cell culture supernatant of claim 63, wherein the recovered supernatant has a rVWF specific ristocetin cofactor activity of at least 50 mU/µg rVWF.

65. The cell culture supernatant of claim 63, wherein the recovered supernatant has a rVWF specific ristocetin cofactor activity of at least 60 mU/µg rVWF.

66. The cell culture supernatant of claim 63, wherein the recovered supernatant has a rVWF specific ristocetin cofactor activity of at least 70 mU/µg rVWF.

67. The cell culture supernatant of claim 63, wherein the recovered supernatant has a rVWF specific ristocetin cofactor activity of at least 80 mU/µg rVWF.

68. The cell culture supernatant according to any one of claims 40 to 67, wherein at least 10% of the rVWF in the supernatant is present in a high molecular weight VWF multimer of more than 10 dimers.

69. The cell culture supernatant according to any one of claims 40 to 67, wherein at least 20% of the rVWF in the supernatant is present in a high molecular weight VWF multimer of more than 10 dimers.

70. The cell culture supernatant according to any one of claims 40 to 67, wherein at least 30% of the rVWF in the supernatant is present in a high molecular weight VWF multimer of more than 10 dimers.

~~71. The cell culture supernatant according to any one of claims 40 to 70, wherein the supernatant contains high molecular weight VWF multimers of 14 to 22 dimers.~~

~~72. The cell culture supernatant according to any one of claims 40 to 71, wherein the composition further comprises recombinant Factor VIII (rFVIII).~~

~~73. The cell culture supernatant of claim 72, wherein the ratio of rVWF to rFVIII is at least 10:1.~~

~~74. The cell culture supernatant of claim 72 or 73, wherein the composition is formulated for pharmaceutical administration.~~

~~75. The cell culture supernatant according to any one of claims 72 to 74, wherein the composition is formulated for intravenous administration.~~

~~76. The cell culture supernatant according to any one of claims 70 to 75, wherein rVWF is co-expressed with rFVIII.~~

~~77. The cell culture supernatant of claim 76, wherein rVWF is further purified away from at least 50% of the rFVIII present in the recovered supernatant.~~

~~78. The cell culture supernatant of claim 77, wherein the ratio of rVWF to rFVIII after the purification step is at least 10:1.~~

~~79. The cell culture supernatant according to any one of claims 40 to 78, wherein rVWF is further enriched.~~