(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 recovered from cells

cultured in a medium, wherein the cell culture supernatant has a final copper concentration between 2.4 µg/L and 20 µg/L, and wherein the $NH4^+$ content of the cell culture supernatant is at a concentration below 4 mM, 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 recovered from cells cultured in a medium, wherein the cell culture supernatant has 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 at a concentration below 4 mM, 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 comprises a hydrolysate.

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, 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 copper supplementing the cell culture media is provided as a copper salt, a copper chelate, or a combination thereof.

9. The composition of claim 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.

10. The composition according to any one of claims 1 to 9, wherein the cells are mammalian cells.

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

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

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

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

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

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

17. The composition according to any one of claims 1 to 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.

18. The composition according to any one of claims 1 to 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.

19. The composition according to any one of claims 1 to 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.

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

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

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

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

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

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