(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.

BRIEF SUMMARY OF ~~INVENTION~~DISCLOSURE

[0020] In certain aspects, the present ~~invention~~disclosure is based on 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.

[0021] In a first aspect, the present ~~invention~~disclosure provides 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

final copper concentration of at least 2.4 μg/L; (c) providing one or more cells comprising a nucleic acid encoding a rVWF protein; (d) culturing the one or more cells in the copper supplemented cell culture media such that rVWF is expressed and excreted from the cells into a culture supernatant; and (e) recovering at least a portion of the culture supernatant, wherein the recovered supernatant has a rVWF specific ristocetin cofactor activity of at least 30 mU/μg rVWF.

[0021a] In one aspect, there is provided a method for producing a recombinant ADAMTS13 (rA13) 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 final copper concentration of from 1.0 μg/L to 6.0 μg/L;

(c) providing one or more cells comprising a nucleic acid encoding a rA13 protein;

(d) culturing the one or more cells in the copper supplemented cell culture media such that rA13 is expressed and excreted from the cells into a culture supernatant, wherein the NH^{4+} content of the cell culture supernatant is maintained at a concentration below 10 mM; and

(e) recovering at least a portion of the culture supernatant.

[0022] In one embodiment of the methods provided above, the method further comprises a step of supplementing the basal cell culture media with a hydrolysate prior to culturing the one or more cells.

[0023] In one embodiment of the methods provided above, the hydrolysate is a plant hydrolysate. In a specific embodiment, the hydrolysate is a soy hydrolysate.

[0024] In one embodiment of the methods provided above, the basal cell culture media is an animal protein free culture media.

[0025] In one embodiment of the methods provided above, the basal cell culture media is a protein free culture media.

[0026] In one embodiment of the methods provided above, the basal cell culture media is a chemically defined culture media.

[0027] In one embodiment of the methods provided above, the final copper concentration of the copper supplemented basal cell culture media is at least 4 μg/L copper.

[0028] In one embodiment of the methods provided above, the final copper concentration of the copper supplemented basal cell culture media is between 2.4 μg/L and 20 μg/L copper.

[0029] In one embodiment of the methods provided above, the copper supplementing the basal cell culture media is provided as a copper salt, a copper chelate, or a combination thereof.

[0030] In one embodiment of the methods provided above, 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.

[0040] In one embodiment of the methods provided above, at least 10% of the rVWF in the supernatant is present in a high molecular weight VWF multimer of more than 10 dimers. In a specific embodiment, at least 15% of the rVWF is present in a high molecular weight VWF multimer of more than 10 dimers. In another specific embodiment, at least 20% of the rVWF is present in a high molecular weight VWF multimer of more than 10 dimers. In another specific embodiment, at least 25% of the rVWF is present in a high molecular weight VWF multimer of more than 10 dimers. In another specific embodiment, at least 30% of the rVWF is present in a high molecular weight VWF multimer of more than 10 dimers.

[0041] In one embodiment of the methods provided above, the supernatant contains high molecular weight VWF multimers of 14 to 22 dimers.

[0042] In one embodiment of the methods provided above, the $NH4^+$ content of the culture supernatant is maintained at a concentration below 10 mM.

[0043] In one embodiment of the methods provided above, the $NH4^+$ content of the culture supernatant is maintained at a concentration below 4 mM.

[0044] In one embodiment of the methods provided above, rVWF is co-expressed with recombinant Factor VIII (rFVIII). In a specific embodiment, the method further comprises a step of purifying rVWF away from at least 50% of the rFVIII present in the recovered supernatant. In one embodiment, the ratio of rVWF to rFVIII after the purification step is at least 10:1.

[0045] In one embodiment of the methods provided above, the method further comprises a rVWF enrichment step.

[0046] In a second aspect, the present ~~invention~~disclosure provides a recombinant Von Willebrand Factor (rVWF) composition prepared by a method provided herein.

[0047] In one embodiment of the compositions provided above, the composition further comprises recombinant Factor VIII (rFVIII). In a specific embodiment, the ratio of rVWF to rFVIII is at least 10:1.

[0048] In one embodiment of the compositions provided above, the composition is formulated for pharmaceutical administration. In a specific embodiment, the composition is formulated for intravenous administration.

[0049] In a third aspect, the present ~~invention~~disclosure provides a cell culture supernatant comprising recombinant Von Willebrand Factor (rVWF), wherein the supernatant is prepared by a method provided herein.

[0050] In a fourth aspect, the present ~~invention~~disclosure provides a cell culture supernatant comprising recombinant Von Willebrand Factor (rVWF), wherein at least 10% of the rVWF in the supernatant is present in a high molecular weight VWF multimer of more than 10 dimers. In a specific embodiment, at least 15% of the rVWF in the supernatant is present in a high molecular weight VWF multimer of more than 10 dimers. In another specific embodiment, at least 20% of the rVWF in the supernatant is present in a high molecular weight VWF multimer of more than 10 dimers. In another specific embodiment, at least 25% of the rVWF in the supernatant is present in a high molecular weight VWF multimer of more than 10 dimers. In another specific embodiment, at least 30% of the rVWF in the supernatant is present in a high molecular weight VWF multimer of more than 10 dimers. In yet another specific embodiment of the supernatants provided above, the supernatant is prepared according to a method provided herein.

[0051] In a fifth aspect, the present ~~invention~~disclosure provides a cell culture supernatant comprising recombinant Von Willebrand Factor (rVWF), wherein the supernatant contains at least 0.4 IU ristocetin cofactor activity per mL. In a specific embodiment, the supernatant contains at least 0.5 IU ristocetin cofactor activity per mL. In another specific embodiment, the supernatant contains at least 0.6 IU ristocetin cofactor activity per mL. In another specific embodiment, the supernatant contains at least 0.7 IU ristocetin cofactor activity per mL. In yet another specific embodiment of the supernatants provided above, the supernatant is prepared according to a method provided herein.

[0052] In a sixth aspect, the present ~~invention~~disclosure provides a method for producing a recombinant ADAMTS13 (rA13) 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 final copper concentration of at least 1.0 μg/L; (c) providing one or more cells comprising a nucleic acid encoding a rA13 protein; (d) culturing the one or more cells in the copper supplemented cell culture media such that rA13 is expressed and excreted from the cells into a culture supernatant; and (e) recovering at least a portion of the culture supernatant, wherein at least 1500 Units

[0075] In one embodiment of the methods provided above, the recovered supernatant has a rA13 specific FRETS-VWF73 activity of at least 800 mU/μg.

[0076] In a preferred embodiment of the methods provided above, the recovered supernatant has a rA13 specific FRETS-VWF73 activity of at least 1200 mU/μg.

[0077] In a more preferred embodiment of the methods provided above, the recovered supernatant has a rA13 specific FRETS-VWF73 activity of at least 1600 mU/μg.

[0078] In one embodiment of the methods provided above, the $NH4^+$ content of the culture supernatant is maintained at a concentration below 10 mM.

[0079] In one embodiment of the methods provided above, the $NH4^+$ content of the culture supernatant is maintained at a concentration below 5 mM.

[0080] In one embodiment of the methods provided above, the $NH4^+$ content of the culture supernatant is maintained at a concentration below 4 mM.

[0081] In one embodiment of the methods provided above, the method further comprises a rA13 enrichment step.

[0082] In a seventh aspect, the present ~~invention~~disclosure provides a cell culture supernatant comprising recombinant ADAMTS13 (rA13), wherein the supernatant is prepared by a method provided herein.

[0083] In an eighth aspect, the present ~~invention~~disclosure provides a cell culture supernatant comprising recombinant ADAMTS13 (rA13), wherein the supernatant contains at least 5 U FRETS-VWF73 activity per mL. In a specific embodiment, the supernatant contains at least 6 U FRETS-VWF73 activity per mL. In another specific embodiment, the supernatant contains at least 7 U FRETS-VWF73 activity per mL. In another specific embodiment, the supernatant contains at least 8 U FRETS-VWF73 activity per mL. In another specific embodiment, the supernatant contains at least 9 U FRETS-VWF73 activity per mL. In another specific embodiment, the supernatant contains at least 10 U FRETS-VWF73 activity per mL. In yet another specific embodiment of the supernatants provided above, the supernatant is prepared according to a method provided herein.

[0084] In a ninth aspect, the present ~~invention~~disclosure provides a cell culture supernatant comprising recombinant ADAMTS13 (rA13), wherein the supernatant contains at least 2 μg rA13 per mL. In a specific embodiment, the supernatant contains at least 3 μg rA13 per mL. In another specific embodiment, the supernatant contains at least 4 μg rA13 per mL. In another specific embodiment, the supernatant contains at least 5 μg rA13 per mL. In another specific embodiment, the supernatant contains at least 6 μg rA13 per mL. In yet another specific embodiment of the supernatants provided above, the supernatant is prepared according to a method provided herein.

[0085] In a tenth aspect, the present ~~invention~~disclosure provides a recombinant ADAMTS13 (rA13) composition prepared by a method according to any one of the methods described above.

[0086] In one embodiment of the compositions provided above, the composition is formulated for pharmaceutical administration. In a specific embodiment, the composition is formulated for intravenous administration.

BRIEF DESCRIPTION OF DRAWINGS

[0087] **Figure 1.** (**1A**) Low resolution (1 %) agarose gel electrophoresis of rVWF expressed in mammalian cell culture in the presence of low (1.0 μg/L) and high (4.3 μg/L) copper concentration, as described in Example 2. Note that culture Day 3 is equivalent to batch day 1 of Table 7 and Table 8. (**1B**) The relative amounts of VWF multimers having 1 to 10 dimers (band number 1) and more than 10 dimers (band number 2), as indicated by the bands defined in Figure 1A, was quantitated by densitometric analysis.

[0088] **Figure 2.** (**2A**) Interval plot of the average rVWF specific activity present in rVWF cell culture supernatants grown at high and low cell densities in the presence of high or low levels of copper. (**2B**) Interval plot of the average NH_4^+ concentration found in rVWF cell culture supernatants grown at high and low cell densities in the presence of high or low levels of copper.

[0089] **Figure 3.** Supernatants from cell cultures expressing recombinant ADAMTS13 in the presence of increasing levels of copper were investigated by SDS-PAGE analysis. rA13 was visualized after SDS-PAGE by (**3A**) silver staining and (**3B**) anti-A13 western blotting.

WHAT IS CLAIMED IS:

1. A method for producing a recombinant ADAMTS13 (rA13) 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 final copper concentration of from 1.0 µg/L to 6.0 µg/L;

(c) providing one or more cells comprising a nucleic acid encoding a rA13 protein;

(d) culturing the one or more cells in the copper supplemented cell culture media such that rA13 is expressed and excreted from the cells into a culture supernatant, wherein the NH^{4+} content of the cell culture supernatant is maintained at a concentration below 10 mM; and

(e) recovering at least a portion of the culture supernatant~~, wherein at least 1500 Units FRETS-VWF73 activity per liter supplemented basal cell culture media per day is present in the recovered culture supernatant~~.

2. The method of claim 1, wherein the basal cell culture media is an animal protein free culture media.

3. The method of claim 1 or 2, wherein the basal cell culture media is a protein free culture media.

4. The method according to any one of claims 1 to 3, wherein the basal cell culture media is a chemically defined culture media.

5. The method according to any one of claims 1 to 4, wherein the final copper concentration of the supplemented basal cell culture media is at least 2 µg/L copper.

6. The method according to any one of claims 1 to 4 wherein the final copper concentration of the supplemented basal cell culture media is at least 4 µg/L copper.

7. The method of claim 1, wherein the final copper concentration of the supplemented basal cell culture media is between 2 µg/L and 4 µg/L copper.