application. In the case of galactosylation and fucosylation, they determine the complement dependent cytotoxicity (CDC) activity and ADCC functions, respectively, that they influence.

The level of β-galactosylation is related to more "mature" glycoforms. Galactose addition is one of the last stages of glycosylation that takes place in the Golgi apparatus before secretion. Terminal galactose is needed for sialylation, which may be the final step in the glycosylation of some proteins. Galactose also serves as a ligand for galactose binding proteins and is the basis of a variety of antigenic responses which are related to carbohydrate content. Galactose has also been shown to impact the conformation of the protein in solution. (Furukawa and Sato, (1999) Biochimica et Biophysica Acta (BBA), 1473 (1), pages 54-86 and Houde et al., (2010) Molecular and Cellular Proteomics, 9(8), pages 1716-1728.

Fucosylation also takes place in the Golgi apparatus as part of the maturation of the protein prior to secretion. If a protein is fucosylated it typically happens before galactosylation in the glycosylation pathway. However, fucosylation is not necessary for galactosylation to proceed (Hossler et al., (2009) Glycobiology, 19(9), pages 936-949).

The influence of glycosylation on bioactivity, pharmacokinetics, immunogenicity, solubility and *in vivo* clearance of therapeutic glycoproteins have made monitoring and control of glycosylation a critical parameter for biopharmaceutical manufacturing. Therefore, methods for manipulating the level of glycan content of therapeutic proteins would be beneficial.

There is a need in the pharmaceutical industry to manipulate and control the level of glycan content of recombinant therapeutic glycoproteins and methods for accomplishing such without significant impact on cell growth, viability and productivity would be useful. The invention provides a method for manipulating the fucosylated glycan content on a recombinant protein by regulating copper and manganese content and pH in cell culture medium.

SUMMARY

In one aspect, the present invention refers to a method for manipulating the fucosylated glycan content on a recombinant protein comprising inoculating a bioreactor with mammalian host cells expressing the recombinant protein, culturing the mammalian host cells in a serum free, chemically defined cell culture medium;

wherein the cell culture medium includes from 10 to 100 ppb copper and from 50 to 1000 nM manganese, at pH 7.0, harvesting the recombinant protein produced by the mammalian host cell, wherein the level of afucosylated glycans on the recombinant protein increases compared to the afucosylated glycan level obtained in the same cell culture medium at a lower pH. Also disclosed herein is a method for manipulating the fucosylated glycan content on a recombinant protein comprising inoculating a bioreactor with host cells expressing the recombinant protein, culturing the host cells in a serum free, chemically defined cell culture medium; wherein the cell culture medium includes

from 10 to 100 ppb copper and from 50 to 1000 nM manganese, at pH 7.0, harvesting the recombinant protein produced by the host cell, wherein the level of afucosylated glycans on the recombinant protein increases compared to the afucosylated glycan level obtained in the same cell culture medium at a lower pH.

In one example the method further comprising an increase in the level of β-galactosylation on the recombinant protein.

In one example the concentration of coper is 100 ppb.

In one example the concentration of manganese is 1000 nM.

In one example the fucosylated glycan content is manipulated to influence the effector function of the recombinant protein.

In one example the method further comprising a temperature shift. In a related example the temperature shift is from 36°C to 31°C. In another related example the temperature shift occurs at the transition between the growth phase and production phase. In yet another related example the temperature shift occurs during the production phase.

In one example the host cell expressing the recombinant protein is cultured in a batch culture, fed-batch culture, perfusion culture, or combinations thereof. In a related example the culture is a perfusion culture. In another related example perfusion comprises continuous perfusion. In another related example rate of perfusion is constant. In another related example the perfusion is performed at a rate of less than or equal to 1.0 working volumes per day. In yet another related example the perfusion is accomplished by alternating tangential flow.

In one example the bioreactor has a capacity of at least 500L.

In one example the bioreactor has a capacity of at least 500L to 2000L.

In one example the bioreactor has a capacity of at least 1000L to 2000L.

In one example the bioreactor is inoculated with at least 0.5 x 10^6 cells/mL.

In one example the serum-free chemically defined cell culture medium is a perfusion cell culture medium.

In one example the host cells are mammalian cells.

In one example the host cells are Chinese Hamster Ovary (CHO) cells.

In one example the recombinant protein is a glycoprotein.

In one example the recombinant protein is selected from the group consisting of a human antibody, a humanized antibody, a chimeric antibody, a recombinant fusion protein, or a cytokine.

In one example the recombinant protein produced by the host cell is purified and formulated into a pharmaceutically acceptable formulation.

In one example is a recombinant protein produced by the method described herein. In a related example the recombinant protein according is purified. In yet another related example the recombinant protein is formulated into a pharmaceutically acceptable formulation.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 Integrated Viable Cell Density (10^6 cell days/ml)

pH 6.85 50 Mn^{2+} 10 Cu^{2+} (gray dashed line with +)

pH 6.85 50 Mn^{2+} 100 Cu^{2+} (gray line with +)

pH 6.85 1000 Mn^{2+} 10 Cu^{2+} (gray dashed line with open circle)

pH 6.85 50 Mn^{2+} 100 Cu^{2+} (gray line with open circle)

pH 7.0 50 Mn^{2+} 10 Cu^{2+} (black dashed line with +)

pH 7.0 50 Mn^{2+} 100 Cu^{2+} (black line with +)

pH 7.0 1000 Mn^{2+} 10 Cu^{2+} (black dashed line with open circle)

pH 7.0 50 Mn^{2+} 100 Cu^{2+} (black line with open circle)

pH appeared to be the only factor impacting cell growth. Concentrations of manganese and copper seemed to have no effect on cell growth.

Fig. 2 Viability (%)

pH 6.85 50 Mn^{2+} 10 Cu^{2+} (gray dashed line with +)

pH 6.85 50 Mn^{2+} 100 Cu^{2+} (gray line with +)

pH 6.85 1000 Mn^{2+} 10 Cu^{2+} (gray dashed line with open circle)

pH 6.85 50 Mn^{2+} 100 Cu^{2+} (gray line with open circle)

pH 7.0 50 Mn^{2+} 10 Cu^{2+} (black dashed line with +)

pH 7.0 50 Mn^{2+} 100 Cu^{2+} (black line with +)

pH 7.0 1000 Mn^{2+} 10 Cu^{2+} (black dashed line with open circle)

pH 7.0 50 Mn^{2+} 100 Cu^{2+} (black line with open circle)

What is claimed is:

1. A method for manipulating the fucosylated glycan content on a recombinant protein comprising

inoculating a bioreactor with mammalian host cells expressing the recombinant protein,

culturing the mammalian host cells in a serum free, chemically defined cell culture medium;

wherein the cell culture medium includes from 10 to 100 ppb copper and from 50 to 1000 nM manganese, at pH 7.0,

harvesting the recombinant protein produced by the mammalian host cell,

wherein the level of afucosylated glycans on the recombinant protein increases compared to the afucosylated glycan level obtained in the same cell culture medium at a lower pH.

2. The method of claim 1, further comprising an increase in the level of β-galactosylation on the recombinant protein.

3. The method according to claim 1, wherein the concentration of copper is 100 ppb.

4. The method according to claim 1, wherein the concentration of manganese is 1000 nM.

5. The method according to claim 1, wherein the fucosylated glycan content is manipulated to influence the effector function of the recombinant protein.

6. The method according to claim 1, further comprising a decreasing temperature shift.

7. The method according to claim 6, wherein the temperature shift is from 36°C to 31°C.

8. The method according to claim 6, wherein the temperature shift occurs at the transition between the growth phase and production phase.

9. The method according to claim 6, wherein the temperature shift occurs during the production phase.

10. The method according claim 1 wherein the mammalian host cell expressing the recombinant protein is cultured in a batch culture, fed-batch culture, perfusion culture, or combinations thereof.

11. The method according to claim 10, wherein the culture is a perfusion culture.

12. The method according to claim 11, wherein perfusion comprises continuous perfusion.

13. The method according to claim 11, wherein the rate of perfusion is constant.

14. The method according to claim 11, wherein the perfusion is performed at a rate of less than or equal to 1.0 working volumes per day.

15. The method according to claim 11, wherein the perfusion is accomplished by alternating tangential flow.

16. The method according to claim 1, wherein the bioreactor has a capacity of at least 500L.

17. The method according to claim 1 wherein the bioreactor has a capacity of at least 500L to 2000L.

18. The method according to claim 1 wherein the bioreactor has a capacity of at least 1000L to 2000L.

19. The method according to claim 1, wherein the bioreactor is inoculated with at least 0.5 x 10^6 cells/mL.

20. The method according to claim 1, wherein the serum-free chemically defined cell culture medium is a perfusion cell culture medium.

21. The method according to claim 1, wherein the mammalian host cells are Chinese Hamster Ovary (CHO) cells.

22. The method according to claim 1, wherein the recombinant protein is a glycoprotein.

23. The method according to claim 1, wherein the recombinant protein is selected from the group consisting of a human antibody, a humanized antibody, a chimeric antibody, a recombinant fusion protein, or a cytokine.

24. The method according to claim 1, wherein the recombinant protein produced by the mammalian host cell is purified and formulated into a pharmaceutically acceptable formulation.