mannose glycoform content by manipulating levels of expression of proteins involved in the N-glycosylation pathway.

SUMMARY

In one aspect, the present disclosure provides a mammalian host cell transfected to regulate the high mannose glycoform content of a recombinant protein of interest during a mammalian cell culture process, the mammalian host cell transfected to overexpress a protein that is involved in an N-glycosylation pathway, wherein the protein involved in the N-glycosylation pathway is selected from the group consisting of N-acetyl-glucosaminyltransferase-1 (encoded by Mgat1), N-acetyl-glucosaminyltransferase-2 (encoded by Mgat2), a UDP-Galactose transporter encoded by Slc35a2, and a combination thereof, and the mammalian host cell transfected to express the recombinant protein of interest during the cell culture process, wherein the high mannose glycoform content of the expressed recombinant protein of interest is less than or equal to 10%.

The present disclosure provides a method for regulating the high mannose glycoform content of a recombinant protein during a mammalian cell culture process comprising transforming a host cell to overexpress a protein that is involved in the N-glycosylation pathway. The disclosure further provides a method for decreasing the high mannose glycoform content of a recombinant protein during the mammalian cell culture process. In one example, the protein is N-acetyl-glucosaminyltransferase-1 (encoded by Mgat1); in another example of the disclosure, the protein is N-acetyl-glucosaminyltransferase-2 (encoded by Mgat2). In a further example of the disclosure, the protein is a UDP-Galactose transporter (encoded by Slc35a2).

Additional examples of the disclosure include transformation of the host cell to overexpress two or more proteins involved in the N-glycosylation pathway, including combinations of the aforementioned proteins. In one example, the host cell is transformed with Mgat1 and Mgat2; in other examples, the host cell is transformed with Mgat1 and Slc35a2, with Mgat2 and Slc35a2, or with Mgat1, Mgat2 and Slc35a2.

The disclosure also provides for transfection of a host cell line that has been previously transfected to express a recombinant protein. In one example, the recombinant protein is a protein comprising an antibody Fc region. Further example includes host cells that express a recombinant protein selected from the group consisting of Fc fusion proteins, antibodies, immunoglobulins, and peptibodies.

In a further example, a host cell is first transfected to overexpress one or more of Mgat1, Mgat2 and Slc35a2, and then is transfected to express a recombinant protein. In one example, the recombinant protein is a protein comprising an antibody Fc

region. Further example includes expression of a recombinant protein selected from the group consisting of Fc fusion proteins, antibodies, immunoglobulins, and peptibodies.

Optionally, the disclosure further comprises a step of harvesting the recombinant protein produced by the cell culture. In a further example the recombinant protein produced by the cell culture is purified and formulated in a pharmaceutically acceptable formulation.

In a further example the high mannose glycoform content of a recombinant protein is decreased compared to that produced by a culture where the cells are not manipulated by transfection to overexpress a protein involved in N-linked glycosylation. In one example the high mannose glycan species is Mannose 5 (Man5). In another example, the high mannose glycan species is Mannose 6 (Man6), Mannose 7 (Man7),

Mannose 8 (including Mannose 8a and 8b; Man8a and 8b, or Mannose 9 (Man9). In a further example the high mannose glycan species comprise a mixture of Man5, Man6, Man7, Man8a, Man8b, and/or Man9.

The disclosure provides a further example in which the high mannose glycoform content of a recombinant protein is reduced. In a further example, the high mannose glycoform content of a recombinant protein is less than or equal to 5%. In another example, the high mannose glycoform content of a recombinant protein is less than or equal to 10%. In a further example, the high mannose glycoform content of a recombinant protein produced by a cell culture of the disclosure is less than 6, 7, 8, 9, or 10 percent. In yet another example, the high mannose glycoform content of a recombinant protein produced by a cell culture of the disclosure is 0.5, 1, 2, 3, 4, or 5%. Further examples include high mannose glycoform content of less than 12%, less than 15%, less than 20%, or less than 30%, 40% or 50%.

Additional examples include the use of a batch or fed-batch culture and the use of a perfusion culture. In one example, the culture is perfused using alternating tangential flow (ATF).

In combination with any of the examples of the disclosure described herein, antifoam may also added into the culture vessel as needed. Alternatively or additionally, 1M Sodium Carbonate or another suitable base is used to maintain pH at the desired setpoint.

As described herein, in one example of the disclosure the cell culture may be maintained by perfusion. In one example perfusion begins on or about day 1 to on or about day 9 of the cell culture. In a related example perfusion begins on or about day 3 to on or about day 7 of the cell culture. In one example perfusion begins when the cells have reached a production phase. In further examples of the disclosure, perfusion is accomplished by alternating tangential flow. In a related example the perfusion is accomplished by alternating tangential flow using an ultrafilter or a microfilter.

A further example of the disclosure provides continuous perfusion; in yet a further example the rate of perfusion is constant. One example of the disclosure provides perfusion performed at a rate of less than or equal to 1.0 working volumes per day. In a related example perfusion is performed at a rate that increases during the production phase from 0.25 working volume per day to 1.0 working volume per day during the cell culture. In another related example perfusion is performed at a rate that reaches 1.0 working volume per day on day 9 to day 11 of the cell culture. In another related example perfusion is performed at a rate that reaches 1.0 working volume per day on day 10 of the cell culture.

In one example the cell culture receives bolus cell culture media feeds prior to days 3 - 7 of the culture.

In yet another example of the disclosure, the cell culture is maintained by fed batch. In one example of a fed batch culture, the culture is fed three times during production. In a further example, the culture is fed on a day between day two and four, on a day between day 5 and 7, and on a day between day 8 and 10. Another example provides a fed batch method in which the culture is fed four times during production. In a still further example, the culture is fed on a day between day two and four, on a day between day 5 and 6, on a day between day 7 and 8, and on a day between day 8 and 10 or later.

According to one example of the disclosure, the mammalian cell culture is established by inoculating the bioreactor with at least 0.5 x 10^6 to 3.0 x 10^6 cells/mL in a serum-free culture media. In an alternate or further example the mammalian cell culture is established by inoculating the bioreactor with at least 0.5 x 10^6 to 1.5 x 10^6 cells/mL in a serum-free culture media.

The disclosure may further comprise a temperature shift during the culture. In one example the temperature shift is from 36°C to 31°C. In one example the disclosure further comprises a temperature shift from 36°C to 33°C. In a related example the temperature shift occurs at the transition between the growth phase and production phase. In a related example the temperature shift occurs during the production phase.

In another example the disclosure further comprises inducing cell growth-arrest by L-asparagine starvation followed by perfusion with a serum-free perfusion media having an L-asparagine concentration of 5 mM or less. In another example the disclosure further comprises inducing cell growth-arrest by perfusion with a serum-free perfusion media having an L-asparagine concentration of 5 mM or less. In a related example the concentration of L-asparagine in the serum-free perfusion media is less than or equal to 5mM. In a related example the concentration of L-asparagine in the serum-free perfusion media is less than or equal to 4.0 mM. In a related example the concentration of L-asparagine in the serum-free perfusion media is less than or equal to 3.0 mM. In a related example the concentration of L-asparagine in the serum-free perfusion media is less than or equal to 2.0 mM. In a related example the concentration of L-asparagine in the serum-free perfusion media is less than or equal to 1.0 mM. In a related example the concentration of L-asparagine in the serum-free perfusion media is 0 mM. In a related example the L-asparagine concentration of the cell culture media is monitored prior to and during L-asparagine starvation.

In yet another example the disclosure comprises that the packed cell volume during a production phase is less than or equal to 35%. In a related example the packed cell volume is less than or equal to 35%. In a related example the packed cell volume is less than or equal to 30%.

In a related example the viable cell density of the mammalian cell culture at a packed cell volume less than or equal to 35% is $10x10^6$ viable cells/ml to $80x10^6$ viable cells/ml. In another example the viable cell density of the mammalian cell culture is $20x10^6$ viable cells/ml to $30x10^6$ viable cells/ml.

In yet another example the bioreactor has a capacity of at least 500L. In yet another example the bioreactor has a capacity of at least 500L to 2000L. In yet another example the bioreactor has a capacity of at least 1000L to 2000L.

In yet another example the mammalian cells are Chinese Hamster Ovary (CHO) cells. In yet another 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.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates clonal variability in doubling time during passaging for the cell lines used for the first set of fed-batch cultures in Example 2. In this Example, cells from a cell line expressing MAb B were transformed to overexpress Mgat1, Mgat2 and/or Slc35A2. M1M2 designates cell lines overexpressing Mgat1 & Mgat2 (individual clones represented by solid circles); M1M2S designates cell lines overexpressing Mgat1, Mgat2 and Slc35a2 (individual clones represented by open triangles); and S designates cell lines overexpressing Slc35A2 (individual clones represented by solid squares). Control cells (individual clones represented by open squares) were transformed with empty vector. The central box spans from the first quartile (Q1) to the third quartile (Q3) and the height of the box is Interquartile range (IQR), the band inside the box is the median, the top whisker extends from Q3 to the largest value falling below Q3+1.5IQR or the maximum value if no value is greater than Q3+1.5IQR. The bottom whisker extends from Q1 to the smallest value falling above Q1-1.5IQR or the minimum value if none is less than Q1-1.5IQR.

Figure 2 illustrates clonal variability in doubling time during passaging for the cell lines used for the second set of fed-batch cells in Example 2. In this Example, cells from a cell line expressing MAb B were transformed to overexpress Mgat1, Mgat2 and/or Slc35A2. M1M2 designates cell lines overexpressing Mgat1 & Mgat2; M1M2S designates cell lines overexpressing Mgat1, Mgat2 and Slc35a2; and S designates cell lines overexpressing Slc35A2. Control cells were transformed with empty vector. Individual clones are designated as described for Figure 1; the parameters for each box are the same as described for Figure 1.

Figure 3 presents the growth shown in terms of viable cell density is comparable for all the overexpressed cell lines (M1M2, M1M2S and S) as compared to control on day 10 of the second fed-batch experiment described in Example 2. The individual values in each box

CLAIMS

What is claimed is:

1. A mammalian host cell transfected to regulate the high mannose glycoform content of a recombinant protein of interest during a mammalian cell culture process,

the mammalian host cell transfected to overexpress a protein that is involved in an N-glycosylation pathway, wherein the protein involved in the N-glycosylation pathway is selected from the group consisting of N-acetyl-glucosaminyltransferase-1 (encoded by Mgat1), N-acetyl-glucosaminyltransferase-2 (encoded by Mgat2), a UDP-Galactose transporter encoded by Slc35a2, and a combination thereof, and

the mammalian host cell transfected to express the recombinant protein of interest during the cell culture process, wherein the high mannose glycoform content of the expressed recombinant protein of interest is less than or equal to 10%.

2. The mammalian host cell of claim 1, wherein the mammalian host cell overexpresses two or more proteins involved in the N-glycosylation pathway, wherein the proteins are selected from the group consisting of Mgat1 and Mgat2; Mgat1 and Slc35a2; Mgat2 and Slc35a2; and Mgat1, Mgat2 and Slc35a2.

3. The mammalian host cell of claim 1, wherein the mammalian host cell overexpresses Mgat1 and Mgat2.

4. The mammalian host cell of claim 1, wherein the high mannose glycoform content of the recombinant protein of interest is less than or equal to 5%.

5. The mammalian host cell of claim 1, wherein the recombinant protein of interest is selected from the group consisting of a protein comprising an antibody Fc region, a Fc fusion protein, an antibody, an immunoglobulin, and a peptibody.

6. The mammalian host cell of claim 1, wherein the recombinant protein of interest is selected from the group consisting of: adalimumab, bevacizumab, infliximab, abciximab, alemtuzumab, bapineuzumab, basiliximab, belimumab, briakinumab, brodalumab, canakinumab, certolizumab pegol, cetuximab, conatumumab, denosumab, eculizumab, etrolizumab, evolocumab, gemtuzumab ozogamicin, golimumab, ibritumomab tiuxetan, labetuzumab, mapatumumab, matuzumab, mepolizumab, motavizumab, muromonab-CD3, natalizumab, nimotuzumab,

ofatumumab, omalizumab, oregovomab, palivizumab, panitumumab, pemtumomab, pertuzumab, ranibizumab, rituximab, rovelizumab, tocilizumab, tositumomab, trastuzumab, ustekinumab, vedolizomab, zalutumumab, and zanolimumab.

7. The mammalian host cell of claim 1, wherein the recombinant protein of interest is selected from the group consisting of: etanercept, abatacept, and belatacept.

8. The mammalian host cell of claim 1, wherein the recombinant protein of interest is an antibody to an antigen selected from the group consisting of: CD2, CD3, CD4, CD8, CD11a, CD14, CD18, CD20, CD22, CD23, CD25, CD33, CD40, CD44, CD52, CD80 (B7.1), CD86 (B7.2), CD147, IL-1alpha, IL-1beta, IL-2, IL-3, IL-7, IL-4, IL-5, IL-8, IL-10, IL-1 receptor, IL-2 receptor, IL-4 receptor, IL-6 receptor, IL-13 receptor, IL-18 receptor subunits, FGL2, PDGF-beta, VEGF, TGF, TGF-beta2, TGFbeta1, EGF receptor, VEGF receptor, hepatocyte growth factor, osteoprotegerin ligand, interferon gamma, B lymphocyte stimulator, C5 complement, IgE, tumor antigen CA125, tumor antigen MUC1, PEM antigen, LCG, HER-2, HER-3, a tumor-associated glycoprotein TAG-72, the SK-1 antigen, integrin alpha 4 beta 7, the integrin VLA-4, integrins, TRAIL receptor 1, TRAIL receptor 2, TRAIL receptor 3, TRAIL receptor 4, RANK, RANK ligand, TNF-alpha, VAP-1, epithelial cell adhesion molecule (EpCAM), intercellular adhesion molecule-3 (ICAM-3), leukointegrin adhesin, the platelet glycoprotein gp IIb/IIIa, cardiac myosin heavy chain, parathyroid hormone, rNAPc2, MHC I, carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), tumor necrosis factor (TNF), CTLA-4, Fc-gamma-1 receptor, HLA-DR 10 beta, HLA-DR antigen, sclerostin, L-selectin, Respiratory Syncytial Virus, human immunodeficiency virus (HIV), hepatitis B virus (HBV), *Streptococcus mutans*, and *Staphylococcus aureus*.

9. The mammalian host cell of claim 1, wherein the mammalian cell is a Chinese Hamster Ovary (CHO) cell.