ovotransferrin. Transferrin is commercially available for use in animal cell culture (e.g. by Sigma-Aldrich, CAS number 11096-37-0). There exist several other iron compounds that are used as transferrin replacement. These exist in II/III forms, as various salts, as hydrated/dehydrated forms. Examples are iron (III) phosphate, iron (III) pyrophosphate, iron (III) nitrate, iron (II) sulfate, iron (III) chloride, iron (II) lactate, ferric (III) citrate, ammonium ferric (III) citrate, iron-dextran, or ethylenediaminetetraacetic acid ferric sodium salt.

We identified iron choline citrate (iron / ferric choline citrate, CAS-Number 1336-80-7, molecular weight Mw = 991,5 g/mol +/- 49.57 g/mol due to 5% crystal water content, iron complex with iron content of about 10.2 - 12.4%, molecule ratio for iron : choline : citrate of 2:3:3, molecule formula $C_{33}H_{57}Fe_2N_3O_{24}$). However, other suitable iron choline citrate complexes are known such as iron : choline : citrate at a ratio of 1:1:1, molecular weight of Mw = 348.11 g/mol. Compared to state of the art iron sources used in commercially available cell culture media such as iron phosphate, iron pyrophosphate or iron citrate, the usage of iron choline citrate contributes to significantly higher product titers. This effect also depends on the iron choline citrate concentration in the media.

SUMMARY

The present disclosure provides a basal cell culture medium and a feed medium with novel amino acid ratios and/or iron choline citrate as iron carrier that improve the performance of mammalian cell culture processes, such as CHO cultivation and protein production processes, in particular product titers (e.g., monoclonal antibody (mAb) titres). Also provided are methods for culturing mammalian cells and producing a protein of interest using said basal cell culture medium and/or feed medium. The disclosure also provides for a medium platform that comprises (i) the basal cell culture medium and (ii) the feed medium. Preferably, both the (basal) cell culture medium and the feed medium are chemically defined.

The disclosure relates to a basal cell culture medium for culturing mammalian cells comprising the following amino acids at a molar ratio relative to isoleucine (mM/mM) of: L-leucine/L-isoleucine of about 1.2-2.2, L-phenylalanine/L-isoleucine of about 0.5-0.9, L-tyrosine/L-isoleucine of about 1.5-2.7, L-threonine/l-isoleucine of about 1.0-1.9, and L-valine/L-isoleucine of about 1.0-1.9, wherein the basal cell culture medium has a total amino acid content of about 25 to 150 mM. In one example the basal cell culture medium further comprises L-lysine at a molar ratio relative to isoleucine of about 1.6-2.9 (mM/mM). The basal cell culture medium may further comprise at least one of the following amino acids at a molar ratio relative to isoleucine (mM/mM) of: L-tryptophan/L-isoleucine of about 0.3-0.5, L-proline/L-isoleucine of about 1.6-3.0; or L-methionine/L-isoleucine of about 0.4-0.7.

Preferably the basal cell culture medium comprises L-tryptophan, L-proline and L-methionine each at said molar ratios as defined above. The basal cell culture medium of the disclosure is a serum-free medium, preferably a chemically defined medium or a chemically defined and protein-free medium. In one example the basal cell culture medium additionally comprises iron choline citrate at a

concentration of about 0.1 to 5.0 mM, about 0.2 to 2.0 mM, about 0.2 to 1.0 mM or about 0.4 to 1.0 mM. In certain examples the basal cell culture medium has a total amino acid content of about 30 to about 130, preferably about 35 to about 120, more preferably about 40 to about 100 mM.

The present disclosure also relates to a basal cell culture medium for culturing mammalian cells comprising iron choline citrate at a concentration of about 0.1 to 5.0 mM, about 0.2 to 2.0 mM, about 0.2 to 1.0 mM or about 0.4 to 1.0 mM. In one aspect, the present disclosure relates to a basal cell culture medium for culturing mammalian cells comprising iron choline citrate at a concentration of 0.1 to 5.0 mM, about 0.2 to 2.0 mM, about 0.2 to 1.0 mM or about 0.4 to 1.0 mM.

The present disclosure relates to a feed medium for culturing mammalian cells comprising the following amino acids at a molar ratio relative to isoleucine (mM/mM) of: L-leucine/L-isoleucine of about 2.3–4.2, L-phenylalanine/L-isoleucine of about 0.6-1.1, L-threonine/l-isoleucine of about 1.3-2.4, and L-valine/L-isoleucine of about 1.1-2.0, wherein the feed medium has a total amino acid content of about 100 to 1000 mM. In one example of the present disclosure the feed medium further comprises the following amino acids at a molar ratio relative to isoleucine (mM/mM) of: L-tyrosine/L-isoleucine of about 0.6-1.1, and/or L-lysine/L-isoleucine of about 1.1-2.1. The feed medium according to the disclosure may further comprise at least one of the following amino acids at a molar ratio relative to isoleucine (mM/mM) of: L-tryptophan/L-isoleucine of about 0.3-0.6, L-proline/L-isoleucine of about 0.9-1.8; or L-methionine/L-isoleucine of about 0.4-0.8. Preferably the feed medium comprises L-tryptophan, L-proline and L-methionine each at said molar ratios as defined above. The feed medium is typically a concentrated feed medium. Preferably the feed medium of the disclosure is a serum-free medium, more preferably a chemically defined medium or a chemically defined and protein-free medium. In one example the feed medium additionally comprises iron choline citrate at a concentration of about 0.4 to 5 mM, about 0.4 to 1.0 mM or about 0.5 to 1.0 mM, preferably about 0.5 to 0.6 mM. In one example the feed medium is characterized by a low salt content, preferably a low salt content of about 100 mM or less and more preferably about 50 mM or less. In certain examples the feed medium of the disclosure has a total amino acid content of about 200 to about 900, preferably about 300 to about 800, more preferably about 400 to about 700 mM.

In another aspect, the present disclosure also relates to a feed medium for culturing mammalian cells comprising iron choline citrate at a concentration of about 0.4 to 5 mM, about 0.4 to 1.0 mM or about 0.5 to 1.0 mM, preferably about 0.5 to 0.6 mM.

In a related aspect the disclosure relates to a medium platform for culturing mammalian cells comprising a) the basal cell culture medium of the disclosure, and b) the feed medium of the disclosure.

The basal cell culture medium and the feed medium of the disclosure are particularly suitable for culturing rodent or human cells, wherein the rodent cell is preferably a Chinese hamster ovary (CHO) cell such as a CHO-K1 cell, a CHO-DG44 cell, a CHO-DUKX B11 cell or a CHO glutamine synthetase (GS) deficient cell, most preferably the cell is a CHO-DG44 or a CHO GS deficient cell.

The disclosure relates to a method of generating a basal cell culture medium comprising: a) providing a basal cell culture medium, and b) adding amino acids at or adjusting the

amino acid ratios to the final molar ratio according to the disclosure. The method may further comprise a step of adding or adjusting as an iron source iron choline citrate at a concentration of about 0.1 to 5.0 mM, about 0.2 to 2.0 mM, about 0.2 to 1.0 mM, or about 0.4 to 1.0 mM.

The disclosure relates to a method of generating a feed medium comprising: providing a feed medium, and adding amino acids at or adjusting the amino acid ratios to the final molar ratios according to the disclosure. The method may further comprise a step of adding or adjusting as an iron source iron choline citrate at a concentration of about 0.4 to 5 mM, about 0.4 to 1.0 mM, or about 0.5 to 1 mM, preferably about 0.5 to 0.6 mM.

In another aspect, the disclosure further relates to a method of culturing a mammalian cell comprising the following steps: a) providing mammalian cells, b) culturing the cells in the basal cell culture medium of the disclosure, and c) optionally adding the feed medium of the disclosure to the basal cell culture medium; wherein the cells are cultured under conditions that allow the cells to proliferate.

In another aspect, the disclosure also relates to a method of producing a protein of interest comprising the following steps: a) providing mammalian cells comprising a gene of interest encoding the protein of interest, b) culturing the cells in the basal cell culture medium of the disclosure, and c) optionally adding the feed medium of the disclosure to the basal cell culture medium, and d) optionally separating and/or isolating and/or purifying said protein of interest from the cell culture; wherein the cells are cultured under conditions that allow expression of the protein of interest. The protein of interest may be a secreted protein, preferably the protein of interest is an antibody or Fc-fusion protein.

The mammalian cell used in any of the methods of the disclosure may be a rodent or human cell, preferably the rodent cell is a Chinese hamster ovary (CHO) cell such as a CHO-K1 cell, a CHO-DG44 cell, a DuxB11 cell or a CHO GS deficient cell, most preferably the cell is a CHO-DG44 or a CHO GS deficient cell. The feed medium used in any of the methods of the disclosure is to be added to the cells cultured in the basal cell culture medium, wherein (a) the feed medium is added at about 10-50 ml/L/day based on the culture starting volume to the basal cell culture medium, (b) the feed medium is added starting on day 0, 1, 2 or 3, and/or (c) the feed medium is added continuously, or as a bolus several times a day, two times a day, once per day, every second day or every third day.

The disclosure relates to a kit of parts comprising the basal cell culture medium of the disclosure and/or the feed medium of the disclosure, and optionally a mammalian cell.

The disclosure further relates to a use of the basal cell culture medium of the disclosure for producing a protein comprising culturing mammalian cells that produce a protein of interest in said medium for a period of time and conditions suitable for cell growth and protein production, harvesting the protein of interest and recovering the protein from the culture medium or cell lysate. The use may further comprise feeding the cells with the feed medium of the disclosure during said culture period.

The disclosure also relates to a use of the feed medium of the disclosure for producing a protein comprising culturing mammalian cells that produce the protein of interest in the basal cell culture medium

of the disclosure for a period of time and conditions suitable for cell growth and protein production, feeding the cells with said feed medium, harvesting the protein of interest and recovering the protein from the culture medium.

In another aspect, the disclosure relates to a use of iron choline citrate as iron carrier in a mammalian cell culture medium, wherein the iron choline citrate is present in the mammalian cell culture medium at a concentration of about 0.2 to 2.0 mM.

DESCRIPTION OF THE FIGURES

Figure 1: RPMI based basal medium with and without optimized amino acid adjustment in a batch experiment at different total amino acid concentrations.

(A-D): CHO2 (CHO-DG44) Rituximab cells were cultured in RPMI based medium 4.1 and medium 5.0 without and with optimized amino acid ratios in duplicates (N=2) with a total amino acid concentration of 44 mM, ◍ culture in medium 5.0, with optimized amino acid ratios (44 mM), ■ culture in medium 4.1, without optimized amino acid ratios (44 mM). Shown are (A) viable cell concentration [$1x10^5$ cell/mL] CHO2 (CHO-DG44) Rituximab cells, (B) viability [%] CHO2 (CHO-DG44) Rituximab cells and (C) product concentration [mg/L] CHO2 (CHO-DG44) Rituximab, (D) Lactate concentration [g/L] CHO2 (CHO-DG44) Rituximab.

(E-H): CHO2 (CHO-DG44) Rituximab cells were cultured in RPMI based medium 4.2 and medium 5.1 without and with optimized amino acid ratios in duplicates (N=2) with a total amino acid concentration of 66 mM, ◍ culture in medium 5.1, with optimized amino acid ratios (66 mM), ■ culture in medium 4.2, without optimized amino acid ratios (66 mM). Shown are (E) viable cell concentration [$1x10^5$ cell/mL] CHO2 (CHO-DG44) Rituximab cells, (F) viability [%] CHO2 (CHO-DG44) Rituximab cells and (G) product concentration [mg/L] CHO2 (CHO-DG44) Rituximab, (H) Lactate concentration [g/L] CHO2 (CHO-DG44) Rituximab.

(I): CHO2 (CHO-DG44) Rituximab cells were cultured in RPMI based medium 4.3 and medium 5.2 without and with optimized amino acid ratios in duplicates (N=2) with a total amino acid concentration of 22 or 36 mM, ■ culture in medium 5.2, with optimized amino acid ratios (22 mM), ► culture in medium 4.3, without optimized amino acid ratios (22 mM), ✚ culture in medium 4.0, without optimized amino acid ratios (36 mM). Shown is (I) product concentration [mg/L] CHO2 (CHO-DG44) Rituximab.

(J): CHO2 (CHO-DG44) Rituximab cells were cultured in RPMI based medium 4.2 and medium 5.2 without and with optimized amino acid ratios in duplicates (N=2) with a total amino acid concentration of 22 and 66 mM, ■ culture in medium 5.2, with optimized amino acid ratios (22 mM), ● culture in medium 4.2, without optimized amino acid ratios (66 mM). Shown is (J) product concentration [mg/L] CHO2 (CHO-DG44) Rituximab.

CLAIMS

1. A basal cell culture medium for culturing mammalian cells comprising iron choline citrate at a concentration of 0.1 to 5.0 mM, about 0.2 to 2.0 mM, about 0.2 to 1.0 mM or about 0.4 to 1.0 mM.

2. The basal cell culture medium of claim 1, further comprising the following amino acids at a molar ratio relative to isoleucine (mM/mM) of:
L-leucine/L-isoleucine of about 1.2-2.2,
L-phenylalanine/L-isoleucine of about 0.5-0.9,
L-tyrosine/L-isoleucine of about 1.5-2.7,
L-threonine/l-isoleucine of about 1.0-1.9, and
L-valine/L-isoleucine of about 1.0-1.9,
wherein the basal cell culture medium has a total amino acid content of about 25 to 150 mM, and wherein the medium is a serum-free medium.

3. The basal cell culture medium of claim 2, further comprising L-lysine at a molar ratio relative to isoleucine of about 1.6-2.9 (mM/mM).

4. The basal cell culture medium of claims 2 or 3, further comprising at least one of the following amino acids at a molar ratio relative to isoleucine (mM/mM) of:
L-tryptophan/L-isoleucine of about 0.3-0.5,
L-proline/L-isoleucine of about 1.6-3.0; or
L-methionine/L-isoleucine of about 0.4-0.7.

5. A feed medium for culturing cells comprising iron choline citrate at a concentration of about 0.4 to 5 mM, about 0.4 to 1.0 mM or about 0.5 to 1.0 mM, preferably about 0.5 to 0.6 mM.

6. The feed medium of claim 5, further comprising the following amino acids at a molar ratio relative to isoleucine (mM/mM) of:
L-leucine/L-isoleucine of about 2.3–4.2,
L-phenylalanine/L-isoleucine of about 0.6-1.1,
L-threonine/l-isoleucine of about 1.3-2.4, and
L-valine/L-isoleucine of about 1.1-2.0,
wherein the feed medium has a total amino acid content of about 100 to 1000 mM, and wherein the medium is a serum-free medium.

7. The feed medium of claim 6, further comprising the following amino acids at a molar ratio relative to isoleucine (mM/mM) of:

L-tyrosine/L-isoleucine of about 0.6-1.1, and/or L-lysine/L-isoleucine of about 1.1-2.1, preferably L-tyrosine/L-isoleucine of about 0.6-1.1, and L-lysine/L-isoleucine of about 1.1-2.1.

8. The feed medium of claims 6 or 7, further comprising at least one of the following amino acids at a molar ratio relative to isoleucine (mM/mM) of:
L-tryptophan/L-isoleucine of about 0.3-0.6,
L-proline/L-isoleucine of about 0.9-1.8; or
L-methionine/L-isoleucine of about 0.4-0.8.

9. A medium platform for culturing mammalian cells comprising:
a) the basal cell culture medium of claims 1 to 4, and
b) the feed medium of claims 5 to 8.

10. A method of culturing a mammalian cell comprising the following steps:
a) providing mammalian cells,
b) culturing the cells in the basal cell culture medium of any one of claims 1 to 4, and
c) optionally adding the feed medium of any one of claims 5 to 8 to the basal cell culture medium;
wherein the cells are cultured under conditions that allow the cells to proliferate.

11. A method of producing a protein of interest comprising the following steps:
a) providing mammalian cells comprising a gene of interest encoding the protein of interest,
b) culturing the cells in the basal cell culture medium of any one of claims 1 to 4, and
c) optionally adding the feed medium of any one of claims 5 to 8 to the basal cell culture medium, and
d) optionally separating and/or isolating and/or purifying said protein of interest from the cell culture;
wherein the cells are cultured under conditions that allow expression of the protein of interest.

12. The method of claim 11, wherein the protein of interest is a secreted protein, preferably the protein of interest is an antibody or Fc-fusion protein.

13. The method of any one of claims 10 to 12, wherein the cell culture is a large-scale cell culture of a working volume of 100 L or more.

14. The cell culture medium of any one of claims 1 to 4, the feed medium of any one of claims 5 to 8, or the method of any one of claims 10 to 13, wherein the mammalian cell is a rodent or human cell, preferably

(a) the rodent cell is a Chinese hamster ovary (CHO) cell such as a CHO-K1 cell, a CHO-DG44 cell, a DuxB11 cell or a CHO GS deficient cell, most preferably the cell is a CHO-DG44 cell or a CHO GS deficient cell; and

(b) the human cell is a PER.C6 cell or a HEK293 cell.

15. Use of iron choline citrate as iron carrier in a mammalian cell culture medium, wherein the iron choline citrate is present in the mammalian cell culture medium at a concentration of about 0.2 to 2.0 mM.