

Claims

1. A method for producing a mammalian milk like product comprising:
 - A) Generating lactocytes mammary-like gland organoids derived from
5 mammalian induced pluripotent stem cells (miPSC);
 - B) Secreting the mammalian milk like product from such lactocytes from
mammalian induced pluripotent stem cells (miPSC).
2. A method according to claim 1 for producing a non-standard human milk like
10 product in vitro comprising:
 - A) Generating lactocytes mammary-like gland organoids derived from human
induced pluripotent stem cells (hiPSC);B) Secreting the human milk like product
from such lactocytes from human induces pluripotent stem cells;Wherein such non standard human milk like product comprises one or more of
15 the nutrients or bioactives selected from the group consisting of proteins,
peptides, lipids, carbohydrates, Vitamins, minerals, choline, myoinositol, L-
carnitine, growth factors, cytokines, probiotics, extracellular vesicles, bioactives
from exosome and secretory IgA.
- 20 3. A method according to claim 1 or 2 which optionally comprises a step C)
whereby the human milk like product of claim 1 is further treated to generate a
modified human milk like product.
- 25 4. A method according to claim 3 wherein step C) is selected in the group consisting
of: a purification step, an isolation process, a fractionation step, an enrichment
process, an enzymatic treatment, the addition of further components and
combinations thereof.

5. A method for producing a human milk like product according to claim 2 to 4 wherein culture conditions according to step A) are adapted to generate lactocytes derived from human induced pluripotent stem cells (hiPSC) capable to secrete a non-standard human milk like product.
6. A method according to anyone of claims 3 to 5 wherein a modified human milk like product is obtained under step C) by adding to the human milk like product of step B) one or more human breast milk components that are not secreted by the lactocytes in step B).
7. A method according to anyone of claims 1 to 6 wherein the lactocytes are part of a mammary gland-like organoid structure generated under step A).
8. A human milk like product which is obtainable according to the method described in anyone of claims 1 to 7.
9. A human milk like product according to claim 8 for use in therapy.
10. Use of a human breast milk like product according to claim 8 as a breast-feeding substitute.
11. A method for producing a human milk like product in vitro comprising:
- A) Generating lactocytes mammary-like gland organoids derived from human induced pluripotent stem cells (hiPSC);
 - B) Secreting the human milk like product from such lactocytes from human induces pluripotent stem cells;

wherein such step A) comprises:

- i) directing hiPSCs to differentiate towards non-neural ectoderm cells by culturing them in an appropriate culture medium, for example MammoCult medium, in an appropriate 3D culture system, for example 3D-suspension condition, for at least 12 days and
- ii) growing the formed mEBs (mammospheres) in an appropriate 3D embedding system, for example a mixed floating gel composed of matrix protein such as Matrigel and/or Collagen I for at least 30 days, for example for 32 days, to generate lactocytes.

12. A method for producing a human milk product according to claim 11 wherein step A)i) is defined as follows:

- i) generation of embryoid bodies (EBs) from hiPSCs by incubation in standard iPSC medium E8 comprising DMEM/F12, L-ascorbic acid-2-phosphate magnesium, sodium selenium, FGF2, insulin, NaHCO₃ and transferrin, TGFβ1 or NODAL or in medium mTeSR™ for two days, and producing mEBs (mammospheres) highly enriched in non-neural ectodermal cells by incubation of EBs in complete MammoCult medium comprising the basal medium, proliferation supplement and supplemented with heparin, and hydrocortisone for 10 days, and wherein
- step A)ii) is distinguished into further substeps and comprises the following steps: ii), iii) and iv):
- ii) incubation of mEBs (mammospheres) in complete EpiCultB medium supplemented with EpiCult proliferation supplement and Parathyroid hormone (pThrP) for 5 days ,
- iii) promotion of branch and alveolar differentiation and mammary cell specification by incubating mEBs (mammospheres) in EpiCultB medium supplemented with EpiCult proliferation supplement, hydrocortisone, insulin, FGF10 and HGF for 20, and
- iv) induction of milk protein expression by incubating mEBs (mammospheres) in EpiCultB medium supplemented with EpiCult proliferation supplement, hydrocortisone, insulin, FBS, prolactin, progesterone and β-estradiol for 7.

13. A method for producing a human milk product according to any of claims 11 or 12 wherein step A)i) is defined as follows:

- 5 i) generation of embryoid bodies (EBs) from hiPSCs by incubation in standard iPSC medium E8 comprising DMEM/F12, L-ascorbic acid-2-phosphate magnesium, sodium selenium, FGF2, insulin, NaHCO₃ and transferrin, TGFβ1 or NODAL for two days, and producing mEBs (mammospheres) highly enriched in non-neural ectodermal cells by incubation of EBs in MammoCultB medium supplemented with MammoCult proliferation supplement, hydrocortisone and heparin for 10 days, and wherein step
- 10 A)ii) is distinguished into further substeps and comprises the following steps ii), iii) and iv):
- ii) embedding the formed mEBs (mammospheres) in a mixture of Matrigel and Collagen I floated in EpiCultB medium supplemented with EpiCult proliferation supplement and Parathyroid hormone (pThrP) for 5 days ,
- 15 iii) promotion of branch and alveolar differentiation and mammary cell specification by incubating embedded mEBs (mammospheres) in EpiCultB medium supplemented with EpiCult proliferation supplement, hydrocortisone, insulin, FGF10 and HGF for 20 days, and
- iv) induction of milk protein expression by incubating mEBs (mammospheres) in
- 20 EpiCultB medium supplemented with EpiCult proliferation supplement, hydrocortisone, insulin, FBS, prolactin, progesterone and β-estradiol for 7 days.

14. A method for producing a human milk like product according to anyone of claims 11 to 13 wherein steps iii) and/or iv) lead to formation of/differentiation into at least

25 breast cells, luminal cells, and basal cells.

15. A method for producing a human milk like product according to claim 14 wherein breast cells express one or more markers selected from the group consisting of: β-

Casein, milk protein, and hormone receptors, luminal cells express one or more all markers selected from the group consisting of: EpCAM, MUC1, CD49F, GATA3, CK8, and CK18, basal cells express one or more markers selected from the group consisting of: CK14, α -smooth muscle actin and P63.

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16. A method for producing a human milk like product according to anyone of claims 11 to 13 wherein after induction of mEBs (mammospheres) in step ii) and/or iv) mammary like gland organoids are obtained, that express one or more markers selected from the group consisting of: β -Casein, milk protein, and hormone receptors, comprising luminal cells that express one or more markers selected from the group consisting of: EpCAM, MUC1, CD49F, GATA3, CK8, CK18, and basal cells that express one or more markers selected from the group consisting of: CK14, α -smooth muscle actin and P63.

15 17. A method for producing a human milk like product according to anyone of claims 11 to 16 wherein the human milk like product is a standard human milk product which comprises: proteins, peptides, lipids (including linoleic acid and alpha-linolenic acid), carbohydrates, Vitamins (including Vitamin A, Vitamin D3, Vitamin E, Vitamin K, Thiamin, Riboflavin, Niacin, Vitamin B6, Vitamin B12, Pantothenic acid, folic acid, 20 Vitamin C and Biotin), minerals (including iron, calcium, phosphorus, magnesium, sodium, chloride, potassium, manganese, iodine, selenium, copper and zinc), choline, myoinositol and L-carnitine, and optionally also comprises at least one bioactive selected from the group consisting of: growth factors, cytokines, probiotics, extracellular vesicles (e.g. milk fat globules and or exosomes), bioactives from exosome 25 (for example miRNA) and secretory IgA.

18. A method for producing a human milk like product according to anyone of claims 11 to 16 wherein the human milk like product is a non-standard human milk product

which comprises one or more of the nutrients or bioactives selected from the group consisting of proteins, peptides, lipids, carbohydrates, Vitamins, minerals, choline, myoinositol, L-carnitine, growth factors, cytokines, probiotics, extracellular vesicles, bioactives from exosome and secretory IgA.

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19. A method according to anyone of claims 11 to 18 which comprises a step C) whereby the human milk like product is further treated to generate a modified human milk like product.

10 20. A method according to claim 19 wherein step C) is selected in the group consisting of: a purification step, an isolation process, a fractionation step, an enrichment process, an enzymatic treatment, the addition of further components and combinations thereof.

15 21. A method for producing a human milk like product according to anyone of claims 11 to 20 wherein culture conditions according to step A) are adapted to generate lactocytes derived from human induced pluripotent stem cells (hiPSC) capable to secrete a non-standard human milk like product.

20 22. A method according to anyone of claims 11 to 21 wherein a modified human milk like product is obtained under step C) by adding to the human milk like product of step B) one or more human breast milk components that are not secreted by the lactocytes in step B).

25 23. A method according to anyone of claims 11 to 22 wherein the lactocytes are part of a mammary gland-like organoid structure generated under step A).

24. A human milk like product which is obtainable according to the method described in anyone of claims 11 to 23.

25. A human milk like product according to claim 24 which comprises bioactives from the group comprising or consisting of: lactose, 6'SL, C-4:0 fatty acid, C-8:0 fatty acid, C-10:0 fatty acid, C-14:0 fatty acid, C-15:0 fatty acid, C-16:0 fatty acid, C-16:1n7 fatty acid, C-17:0 fatty acid, C-18:0 fatty acid, C-18:1 n9 fatty acid, C-18:1 fatty acid, C-18:2 n6 fatty acid, C-20:0 fatty acid, C-20:1 n9 fatty acid, C-18:3 n3 fatty acid, C-22:0 fatty acid, lactoferrin, albumin, prolactin, Alpha S1-casein, Hemoglobin subunit beta, Hemoglobin subunit alpha, α -lactalbumin, Alpha-2-macroglobulin, β -casein, bile salt-activated lipase, κ -casein, lactadherin, CD14, fatty acid synthase, IgA, plgR, Serum albumin, Xanthine dehydrogenase, exosomes, miR-21-5p, miR-181a-5p, miR-30d-5p, miR-30b-5p, miR-22-3p, miR-146b-3p, miR-30c-5p, miR-30a-5p, miR-30e-5p and miR-148b-3p.

26. A human milk like product according to claim 24 or 25 which is a standard human milk like product which comprises: proteins, peptides, lipids, carbohydrates, Vitamins, minerals, choline, myoinositol and L-carnitine, and optionally also comprises at least one bioactive selected from the group consisting of: growth factors, cytokines, probiotics, extracellular vesicles, bioactives from exosome (for example miRNA) and secretory IgA.

27. A human milk like product according to claim 24 or 25 which is a non -standard human milk like product which comprises one or more of the nutrients or bioactives selected from the group consisting of proteins, peptides, lipids, carbohydrates, Vitamins, minerals, choline, myoinositol, L-carnitine, growth factors, cytokines, probiotics, extracellular vesicles, bioactives from exosome and secretory IgA.

28. A human milk like product according to anyone of claims 24 to 27 for use in therapy.

29. Use of a human breast milk like product according to anyone of claim 24 to 27 as a breast-feeding substitute.