Claims

1. A method for producing a human milk like product comprising:

A) generating lactocytes mammary-like gland organoids derived from human induced pluripotent stem cells (hiPSC) by culturing iPSCs in 3D-suspension conditions to form mammospheres and culturing said mammospheres in 3D-suspension conditions for at least 10 days to generate lactocytes; and

B) secreting the human milk like product from such lactocytes from human induced pluripotent stem cells (hiPSC).

2. The method according to claim 1, wherein step A) is conducted for at least 42 days.

3. The method according to claim 1 or claim 2, wherein step A) is conducted in a bioreactor.

4. The method according to any one of claims 1 to 3, 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, $NaHCO_3$ and transferrin, TGFβ1 or NODAL or in medium comprising DMEM/F12, BSA, sodium bicarbonate, thiamine hydrochloride, reduced glutathione, L-ascorbic acid 2-phosphate magnesium, γ-aminobutyric acid, lithium chloride, pipecolic acid, selenium, trace minerals, human insulin, bFGF, TGFβ, defined lipid concentrate, non-essential amino acids, L-glutamine, sodium hydroxide and sodium chloride for two days, and producing mEBs (mammospheres) comprising non-neural ectodermal cells by incubation of EBs in complete medium comprising 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 medium supplemented with 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 conditioned medium supplemented with proliferation supplement, hydrocortisone, insulin, FGF10 and HGF for 20 days, and

iv) induction of milk protein expression by incubating mEBs (mammospheres) in a conditioned medium supplemented with proliferation supplement, hydrocortisone, insulin, FBS, prolactin, progesterone and β-estradiol for 7 days.

5. The method according to claim 4, wherein steps iii) and/or iv) lead to formation of/differentiation into at least breast cells, luminal cells, and basal cells.

6. The method according to claim 5, 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.

7. The method according to claim 4, 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.

8. The method according to any one of claims 4 to 7, wherein the human milk like product 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, 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 (for example miRNA) and secretory IgA.

9. The method according to any one of claims 1 to 8, further comprising a step C) whereby the human milk like product is further treated to generate a modified human milk like product.

10. The method according to claim 9, wherein step C) is selected from 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.

11. The method according to claim 9 or 10, wherein the 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).

12. The method according to any one of claims 1 to 11, wherein the culture conditions according to step A) are adapted to generate lactocytes derived from human induced pluripotent stem cells (hiPSC) capable of secreting only one or a portion of bioactives and nutrients found in the human milk like product, optionally wherein the bioactives and nutrients are 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.

13. A human milk like product which is obtainable according to the method described in any one of claims 1 to 12.

14. The human milk like product according to claim 13 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, haemoglobin subunit beta, haemoglobin subunit alpha, α-lactalbumin, alpha-2-macroglobulin, β-casein, bile salt-activated lipase, κ-casein, lactadherin, CD14, fatty acid synthase, IgA, pIgR, 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.

15. The human milk like product according to claim 13 or 14 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.

16. A human milk like product according to any one of claims 13 to 15 for use in therapy.

17. Use of a human breast milk like product according to any one of claims 13 to 15 as a breast-feeding substitute.