

CLAIMS

What is claimed is:

1. A method of producing an isolated cultured milk product from mammary cells, the method comprising:
 - a. culturing a cell construct in a bioreactor under conditions which produce the cultured milk product, said cell construct comprising:
 - i. a three-dimensional scaffold having an exterior surface, an interior surface defining an interior cavity, and a plurality of pores extending from the interior surface to the exterior surface;
 - ii. a matrix material disposed on the exterior surface of the three-dimensional scaffold;
 - iii. a culture media disposed within the interior cavity and in fluidic contact with the internal surface;
 - iv. a plurality of plasma cells disposed on the matrix material, and
 - v. a confluent monolayer of polarized mammary cells disposed on the plurality of plasma cells, wherein the mammary cells are selected from the group consisting of: mammary epithelial cells, mammary myoepithelial cells, and mammary progenitor cells, wherein the polarized mammary cells comprise an apical surface and a basal surface; and
 - b. isolating the cultured milk product.
2. The method of claim 1, wherein the monolayer of polarized mammary cells is at least 70% confluent, at least 80% confluent, at least 90% confluent, at least 95% confluent, at least 99% confluent, or 100% confluent.
3. The method of claim 1, wherein at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or 100% of the mammary cells are polarized in the same orientation.
4. The method of claim 1, wherein the cultured milk product comprises secretory IgA (sIgA).
5. The method of claim 1, wherein the bioreactor comprises an apical compartment that is substantially isolated from the internal cavity of the cell construct.
6. The method of claim 1, wherein the basal surface of the mammary cells is in fluidic contact with the culture media.
7. The method of claim 5, wherein the apical compartment is in fluidic contact with the apical surface of the mammary cells.
8. The method of claim 7, wherein the cultured milk product is secreted from the apical surface of the mammary cells into the apical compartment.

9. The method of claim 1, wherein the culture media substantially does not contact the cultured milk product.
10. The method of claim 1, wherein total cell density of mammary cells within the bioreactor is at least 10^{11} ; and alternatively wherein total surface area of mammary cells within the bioreactor is at least 1.5 m^2 .
11. The method of claim 1, wherein total cell density of plasma cells in the bioreactor is about 200 to 500 plasma cells per mm^2 .
12. The method of claim 1, wherein the culturing is carried out at a temperature of about 27°C to about 39°C .
13. The method of claim 1, wherein the culturing is carried out at an atmospheric concentration of CO_2 of about 4% to about 6%.
14. A cell construct, comprising:
 - a. a three dimensional scaffold having an exterior surface, an interior surface defining an interior cavity/basal chamber, and a plurality of pores extending from the interior surface to the exterior surface;
 - b. a matrix material disposed on the exterior surface of the three-dimensional scaffold;
 - c. a culture media disposed within the interior cavity/basal chamber and in fluidic contact with the internal surface;
 - d. a plurality of plasma cells disposed on the matrix material; and
 - e. an at least 70% confluent monolayer of polarized mammary cells disposed on the plurality of plasma cells, wherein the mammary cells are selected from the group consisting of: mammary epithelial cells, mammary myoepithelial cells, and mammary progenitor cells.
15. The cell construct of claim 14, wherein the polarized mammary cells comprise an apical surface and a basal surface.
16. The cell construct of claim 15, wherein the basal surface of the mammary cells is in fluidic contact with the culture media.
17. The cell construct of claim 14, wherein at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or 100% of the mammary cells are polarized in the same orientation.
18. The cell construct of claim 14, wherein the monolayer of polarized mammary cells is at least 70% confluent, at least 80% confluent, at least 90% confluent, at least 95% confluent, at least 99% confluent, or 100% confluent.

19. The cell construct of claim 14, wherein the mammary cells comprise a constitutively active prolactin receptor protein.
20. The cell construct of claim 14, wherein the culture medium comprises prolactin.
21. A cultured milk product, comprising: Beta-casein, Alpha-lactalbumin, Kappa-casein, Alpha-S1-casein, Lactoferrin, Lactadherin, Lysozyme, C12:0 (lauric acid), C14:0 (myristic acid), C16:0 (palmitic acid), Lactose, Glucose, 3'-SL, and 6'-SL;
 wherein the cultured milk product does not comprise or is substantially free of persistent organic pollutants (POPs), heavy metals, prescription drugs, recreational drugs, allergens, cells, hormones, or virus;
 provided that the cultured milk product may comprise a human mammary epithelial cell (hMEC) or a plasma cell (PC).
22. The cultured milk product of claim 21, further comprising: C18:2n-6 (linoleic acid, LA), C18:3n-3 (alpha-linolenic acid, ALA), Butrophilin, Osteopontin, Mucin-4, Mucin-1, C8:0 (caprylic acid), C10:0 (capric acid), C13:0 (tridecylic acid C16:1 (palmitoleic acid), C20:3n-6 (dihomo-gama-linolenic acid, DGLA), C20:4n-6 (arachadonic acid, AA), C20:5n-3 (eicosopentanoic acid, EPA), C22:6n-3 (docosahexaenoic acid, DHA), C22:1n9 (erucic acid), C24:1 (nervonic acid), LNT, or LNT.
23. The cultured milk product of claim 21, further comprising at least one oxylin metabolite of a polyunsaturated fatty acid or at least one endocannabinoid.
24. The cultured milk product of claim 23, wherein the oxylin metabolite is 9-hydroxyoctadecadienoic acid (9-HODE); 13-hydroxyoctadecadienoic acid (13-HODE); 5,15-dihydroxyeicosatetraenoic acid (5,15-DiHETE); 17,18-dihydroxyeicosatetraenoic acid (17,18-DiHETE); 8,9-Dihydroxyicosatrienoic acid (8,9-DiHETrE); 11,12-Dihydroxyicosatrienoic acid (11,12-DiHETrE); 9,10-dihydroxyoctadecenoic acid (9,10-DiHOME); 12,13-dihydroxyoctadecenoic acid (12,13-DiHOME); 14(15)-epoxyeicosatrienoic acid (14(15)-EpETrE); 19(20)-epoxydocosapentanoic acid (19(20)-EpDPE); 17-hydroxydocosahexaenoic acid (17-HDoHE); 5-hydroxyeicosatetraenoic acid (5-HETE); 8-hydroxyeicosatetraenoic acid (8-HETE); 9-hydroxyeicosatetraenoic acid (9-HETE); 11-hydroxyeicosatetraenoic acid (11-HETE); 12-hydroxyeicosatetraenoic acid (12-HETE); 9-hydroxyoctadecatrienoic acid (9-HOTrE); 9-oxo-octadecadienoic acid (9-oxo-ODE); or 13-oxo-octadecadienoic acid (13-oxo-ODE); 17,18-epoxyeicosatetraenoic acid (17(18)-EpETE); 6-Keto-prostaglandin F1alpha (6-keto-PGF1a); or 15(S)-Hydroxyeicosatrienoic acid (15(S)-HETrE).
25. The cultured milk product of claim 23, wherein the endocannabinoid is anandamide or 2-arachidonoylglycerol (2-AG).

26. The cultured milk product of claim 21, further comprising secretory IgA (sIgA).
27. The cultured milk product of claim 21, wherein the cultured milk product does not comprise or is substantially free of persistent organic pollutants (POPs).
28. The cultured milk product of claim 27, wherein the cultured milk product does not comprise or is substantially free of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs) and pesticides.
29. The cultured milk product of claim 28, wherein the cultured milk product does not comprise or is substantially free of DDT.
30. The cultured milk product of claim 21, wherein the cultured milk product does not comprise or is substantially free of heavy metals.
31. The cultured milk product of claim 30, wherein the cultured milk product does not comprise or is substantially free of mercury, lead, arsenic, cadmium, nickel, chromium, cobalt, or zinc.
32. The cultured milk product of claim 21, wherein the cultured milk product does not comprise or is substantially free of pharmaceutical or recreational drugs.
33. The cultured milk product of claim 32, wherein the cultured milk product does not comprise or is substantially free of chemotherapeutics, antidepressants, or anti-anxiety medications.
34. The cultured milk product of claim 32, wherein the cultured milk product does not comprise or is substantially free of benzodiazepines.
35. The cultured milk product of claim 32, wherein the cultured milk product does not comprise or is substantially free of lithium, carbamazepine, chlorpromazine, and clozapine.
36. The cultured milk product of claim 32, wherein the cultured milk product does not comprise or is substantially free of alcohol, cannabis, opioids, phencyclidine, or cocaine.
37. The cultured milk product of claim 21, wherein the cultured milk product does not comprise or is substantially free of allergens.
38. The cultured milk product of claim 37, wherein the cultured milk product does not comprise or is substantially free of ovomucoid, ovalbumin, conalbumin, arachin 6, arachin 3, conarachin, Arah1, and arachin Arah2.
39. The cultured milk product of claim 21, wherein the cultured milk product does not comprise or is substantially free of human stem cells, human immune cells, or bacterial cells, provided that the cultured milk product comprises one or more human mammary epithelial cells (hMECs) or plasma cells (PCs).

40. The cultured milk product of claim 39, wherein the cultured milk product does not comprise or is substantially free of myoepithelial cells, myeloid precursor cells, neutrophils, granulocytes, or T cells.
41. The cultured milk product of claim 39, wherein the cultured milk product does not comprise or is substantially free of Staphylococcus, Acinetobacter, Streptococcus, Pseudomonas, Lactococcus, Enterococcus or Lactobacillus.
42. The cultured milk product of claim 21, wherein the cultured milk product does not comprise or is substantially free of virus.
43. The cultured milk product of claim 42, wherein the cultured milk product does not comprise or is substantially free of hepatitis B virus, hepatitis C virus, cytomegalovirus, West Nile virus, human T-cell lymphotropic virus, and human immunodeficiency virus.
44. The cultured milk product of claim 21, wherein the cultured milk product does not comprise or is substantially free of hormones.
45. The cultured milk product of claim 44, wherein the cultured milk product does not comprise or is substantially free of leptin, ghrelin, adiponectin, thyroxine (T4), triiodothyronine (T3) thyroid-stimulating hormone (TSH), epidermal growth factor, beta-endorphin, relaxin, cortisol, or erythropoietin.
46. A cultured milk product, comprising: Beta-casein; Alpha-lactalbumin; Kappa-casein; Alpha-S1-casein; Lactoferrin; Butrophilin; Osteopontin; Lactadherin; Lysozyme; Mucin-4; Mucin-1; C8:0 (caprylic acid); C10:0 (capric acid); C12:0 (lauric acid); C13:0 (tridecylic acid); C14:0 (myristic acid); C16:0 (palmitic acid); C16:1 (palmitoleic acid); C18:2n-6 (linoleic acid, LA); C18:3n-3 (alpha-linolenic acid, ALA); C20:3n-6 (dihomo-gama-linolenic acid, DGLA); C20:4n-6 (arachadonic acid, AA); C20:5n-3 (eicosopentanoic acid, EPA); C22:6n-3 (docosahexaenoic acid, DHA); C22:1n9 (eruiic acid); C24:1 (nervonic acid); Lactose; Glucose; 3'-SL; 6'-SL; LNT; LNnT; 9-hydroxyoctadecadienoic acid (9-HODE); 13-hydroxyoctadecadienoic acid (13-HODE); 5,15-dihydroxyeicosatetraenoic acid (5,15-DiHETE); 17,18-dihydroxyeicosatetraenoic acid (17,18-DiHETE); 8,9-Dihydroxyicosatrienoic acid (8,9-DiHETrE); 11,12-Dihydroxyicosatrienoic acid (11,12-DiHETrE); 9,10-dihydroxyoctadecenoic acid (9,10-DiHOME); 12,13-dihydroxyoctadecenoic acid (12,13-DiHOME); 14(15)-epoxyeicosatrienoic acid (14(15)-EpETre); 19(20)-epoxydocosapentanoic acid (19(20)-EpDPE); 17-hydroxydocosahexaenoic acid (17-HDoHE); 5-hydroxyeicosatetraenoic acid (5-HETE); 8-hydroxyeicosatetraenoic acid (8-HETE); 9-hydroxyeicosatetraenoic acid (9-HETE); 11-hydroxyeicosatetraenoic acid (11-HETE); 12-hydroxyeicosatetraenoic acid (12-HETE); 9-hydroxyoctadecatrienoic acid (9-HOTrE); 9-oxo-octadecadienoic acid (9-oxo-ODE); or 13-oxo-octadecadienoic acid (13-oxo-ODE); 17,18-epoxyeicosatetraenoic acid (17(18)-EpETE); 6-Keto-

prostaglandin F1alpha (6-keto-PGF1a); or 15(S)-Hydroxyeicosatrienoic acid (15(S)-HETrE); and sIgA;

wherein the cultured milk product does not comprise or is substantially free of persistent organic pollutants (POPs), heavy metals, prescription drugs, recreational drugs, allergens, cells, hormones, or virus;

provided that the cultured milk product may comprise human mammary epithelial cells or plasma cells.

47. The cultured milk product of any one of claims 21-46, wherein the cultured milk product further comprises a preservative, stabilizer, antioxidant, emulsifier, or thickener.
48. The cultured milk product of claim 47, wherein the cultured milk product further comprises lecithin.
49. The cultured milk product of claim 47, wherein the cultured milk product further comprises carrageenan.
50. The cultured milk product of claim 47, wherein the cultured milk product further comprises beta carotene.
51. The cultured milk product of claim 47, wherein the cultured milk product further comprises vitamin E.
52. The cultured milk product of claim 47, wherein the cultured milk product further comprises ascorbyl palmitate or ascorbic acid. I
53. The cultured milk product of claim 47, wherein the cultured milk product further comprises lecithin, ascorbyl palmitate and vitamin E
54. The cultured milk product of any one of claims 21-53, wherein the cultured milk product is dehydrated, freeze-dried, or frozen.
55. The cultured milk product of any one of claims 21-54, wherein the cultured milk product is food-grade.
56. A method of feeding a human subject in need thereof, comprising administering to the subject a cultured milk product according to any one of claims 21-55.
57. The method of claim 56, wherein the human subject is an infant.
58. The method of claim 56, wherein the human subject is immuno-compromised.
59. The method of claim 58, wherein the human subject has a disease selected from: severe combined immunodeficiency (SCID), HIV/AIDS, a cancer, or an autoimmune disease.

60. The method of claim 59, wherein the subject has lupus or diabetes (for example, Type I diabetes or Type II diabetes).
61. The method of claim 58, wherein the human subject is an organ or bone marrow transplant recipient.
62. The method of claim 56, wherein the human subject is malnourished.
63. The method of claim 56, wherein the human subject has malabsorption syndrome.
64. The method of claim 56, wherein the human subject has wasting syndrome.
65. The method of claim 56, wherein the human subject is geriatric.
66. A method of treating or preventing a microbial infection in a subject in need thereof, comprising administering to the subject a cultured milk product of claim 26 or 46.
67. The method of claim 66, wherein the infection is a bacterial infection, fungal infection or parasitic infection.
68. The method of claim 67, wherein the bacterial infection is an infection of *E. coli*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella*, *Shigella*, *Campylobacter*, *Staphylococcus aureus*, *Helicobacter pylori*, *C. difficile*, or *Vibrio cholerae*.
69. The method of claim 67, wherein the parasitic infection is an infection of *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium* spp., or *Cystoisospora belli*.
70. The method of claim 66, wherein the microbial infection is a gastrointestinal infection.
71. The method of claim 70, wherein the gastrointestinal infection is gastroenteritis.
72. The method of claim 66, wherein the microbial infection is Candidiasis.
73. A method of producing isolated sIgA from mammary cells, the method comprising:
 - a. culturing a cell construct in a bioreactor under conditions which produce a cultured milk product, said cell construct comprising:
 - i. a three-dimensional scaffold having an exterior surface, an interior surface defining an interior cavity, and a plurality of pores extending from the interior surface to the exterior surface;
 - ii. a matrix material disposed on the exterior surface of the three-dimensional scaffold;
 - iii. a culture media disposed within the interior cavity and in fluidic contact with the internal surface;
 - iv. a plurality of plasma cells disposed on the matrix material; and

- v. a confluent monolayer of polarized mammary cells disposed on the plurality of plasma cells, wherein the mammary cells are selected from the group consisting of: mammary epithelial cells, mammary myoepithelial cells, and mammary progenitor cells, wherein the polarized mammary cells comprise an apical surface and a basal surface; and
 - b. isolating the sIgA from the cultured milk product.
74. The method of claim 73, wherein the monolayer of polarized mammary cells is at least 70% confluent, at least 80% confluent, at least 90% confluent, at least 95% confluent, at least 99% confluent, or 100% confluent.
75. The method of claim 73, wherein at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or 100% of the mammary cells are polarized in the same orientation.
76. The method of claim 73, wherein the cultured milk product comprises secretory IgA (sIgA).
77. The method of claim 73, wherein the bioreactor comprises an apical compartment that is substantially isolated from the internal cavity of the cell construct.
78. The method of claim 73, wherein the basal surface of the mammary cells is in fluidic contact with the culture media.
79. The method of claim 77, wherein the apical compartment is in fluidic contact with the apical surface of the mammary cells.
80. The method of claim 79, wherein the cultured milk product is secreted from the apical surface of the mammary cells into the apical compartment.
81. The method of claim 73, wherein the culture media substantially does not contact the cultured milk product.
82. The method of claim 73, wherein total cell density of mammary cells within the bioreactor is at least 10^{11} ; and alternatively wherein total surface area of mammary cells within the bioreactor is at least 1.5 m^2 .
83. The method of claim 73, wherein total cell density of plasma cells in the bioreactor is about 200 to 500 plasma cells per mm^2 .
84. The method of claim 73, wherein the culturing is carried out at a temperature of about 27°C to about 39°C .
85. The method of claim 73, wherein the culturing is carried out at an atmospheric concentration of CO_2 of about 4% to about 6%.
86. A method of treating or preventing a microbial infection in a subject in need thereof, comprising administering to the subject a pharmaceutical composition comprising (a) sIgA and (b) a pharmaceutically acceptable excipient,

wherein the sIgA is manufactured by a method according to any one of claims 73-85.

87. The method of claim 86, wherein the human subject is an infant.
88. The method of claim 86, wherein the human subject is immuno-compromised.
89. The method of claim 88, wherein the human subject has a disease selected from: severe combined immunodeficiency (SCID), HIV/AIDS, a cancer, or an autoimmune disease.
90. The method of claim 89, wherein the subject has lupus or diabetes (for example, Type I diabetes or Type II diabetes).
91. The method of claim 88, wherein the human subject is an organ or bone marrow transplant recipient.
92. The method of claim 86, wherein the human subject is malnourished.
93. The method of claim 86, wherein the human subject has malabsorption syndrome.
94. The method of claim 86, wherein the human subject has wasting syndrome.
95. The method of claim 86, wherein the human subject is geriatric.
96. The method of claim 86, wherein the human subject has cystic fibrosis, COPD, or non-CF bronchiectasis.
97. The method of claim 86, wherein the infection is a bacterial infection, fungal infection or parasitic infection.
98. The method of claim 96, wherein the bacterial infection is an infection of *E. coli*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella*, *Shigella*, *Campylobacter*, *Staphylococcus aureus*, *Helicobacter pylori*, *C. difficile*, or *Vibrio cholerae*.
99. The method of claim 96, wherein the parasitic infection is an infection of *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium* spp., or *Cystoisospora belli*.
100. The method of claim 86, wherein the microbial infection is a gastrointestinal infection.
101. The method of claim 99, wherein the gastrointestinal infection is gastroenteritis.
102. The method of claim 86, wherein the microbial infection is Candidiasis.
103. The method of claim 86, wherein the microbial infection is a respiratory infection.
104. The method of claim 103, wherein the respiratory infection is pneumonia, bronchitis, Aspergillosis, or Cryptococcosis.
105. The method of claim 103, wherein the respiratory infection is an infection by *B. cepacia*, *P. aeruginosa*, *S. aureus*, *Aspergillus*, *Cryptococcus*, or *Pneumocystis*.

106. The method of claim 86 or any one of 103-105, wherein the composition is formulated for inhalation.
107. The method of claim 106, wherein the composition is a powder.
108. The method of claim 106, wherein the composition is formulation for administration by a nebulizer.
109. A pharmaceutical composition, comprising:
 - a. sIgA manufactured by a method disclosed herein; and
 - b. a pharmaceutically acceptable excipient.
110. The pharmaceutical composition of claim 109, wherein the pharmaceutically acceptable excipient is a stabilizer, a surfactant, a buffer or tonicity agent.
111. The pharmaceutical composition of claim 109, wherein the pharmaceutically acceptable excipient is sucrose, trehalose, mannitol, sorbitol, histidine, arginine, glycine, polysorbate 20, polysorbate 80, poloxamer 188, edetic acid/or edetate salts (e.g., EDTA), glutathione, metacresol, phenol, benzyl alcohol, benzalkonium chloride, methionine or cysteine.