

MILK PRODUCT COMPOSITIONS

CROSS-REFERENCE

[0001] This application claims the benefit of priority from U.S. Provisional Application No. 63/030,149 filed on May 26, 2020, U.S. Provisional Application No. 63/200,480 filed on March 9, 2021, and U.S. Application No. 17/301,216 filed on March 29, 2021, the contents of each are incorporated herein by reference in their entireties.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 24, 2021, is named BMQ_002WO_SL.txt and is 29,447 bytes in size.

FIELD

[0003] This disclosure relates to milk product compositions that comprise protein, lipid, and oligosaccharide components and component concentrations that mimic human breast milk as produced by a lactating female, which milk compositions are produced *in vitro* and/or *ex vivo* from cultured mammary cells.

BACKGROUND

[0004] Milk is a staple of the human diet, both during infancy and throughout life. The American Academy of Pediatrics and World Health Organization recommend that infants be exclusively breastfed for the first 6 months of life, and consumption of dairy beyond infancy is a mainstay of human nutrition, representing a 700 billion dollar industry worldwide. However, lactation is a physiologically demanding and metabolically intensive process that can present biological and practical challenges for breastfeeding mothers, and milk production is associated with environmental, social, and animal welfare impacts in agricultural contexts.

[0005] The possibility of using mammalian cell culture to produce food has gained increasing interest in recent years, with the development of several successful prototypes of meat and sea food products from cultured muscle and fat cells (Stephens et al. 2018 *Trends Food Sci Technol.* 78:155-166). Additionally, efforts are underway to commercialize the production of egg and milk proteins using microbial expression systems. However, this fermentation-based process relies on the genetically engineered expression and purification of individual components and is unable to reproduce the full molecular profile of milk or dairy.

SUMMARY OF THE DISCLOSURE

[0006] Disclosed herein, in certain embodiments, are methods of producing an isolated cultured milk product from mammary cells, the methods 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. In some embodiments, 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. In some embodiments, 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. In some embodiments, the cultured milk product comprises secretory IgA (sIgA). In some embodiments, the bioreactor comprises an apical compartment that is substantially isolated from the internal cavity of the cell construct. In some embodiments, the basal surface of the mammary cells is in fluidic contact with the culture media. In some embodiments, the apical compartment is in fluidic contact with the apical surface of the mammary cells. In some embodiments, the cultured milk product is secreted from the apical surface of the mammary cells into the apical compartment. In some embodiments, the culture media substantially does not contact the cultured milk product. In some embodiments, 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 . In some embodiments, total cell density of plasma cells in the bioreactor is about 200 to 500 plasma cells per mm^2 . In some embodiments, the culturing is carried out at a temperature of about 27°C to about 39°C . In some embodiments, the culturing is carried out at an atmospheric concentration of CO_2 of about 4% to about 6%.

[0007] Disclosed herein, in certain embodiments, are cell constructs, 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. In some embodiments, the polarized mammary

cells comprise an apical surface and a basal surface. In some embodiments, the basal surface of the mammary cells is in fluidic contact with the culture media. In some embodiments, 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. In some embodiments, 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. In some embodiments, the mammary cells comprise a constitutively active prolactin receptor protein. In some embodiments, the culture medium comprises prolactin.

[0008] Disclosed herein, in certain embodiments, are cultured milk products, 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 composition 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 composition may comprise a human mammary epithelial cell (hMEC) or a plasma cell (PC). In some embodiments, a cultured milk product disclosed herein further comprises: 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. In some embodiments, a cultured milk product disclosed herein further comprises at least one oxylipin metabolite of a polyunsaturated fatty acid or at least one endocannabinoid. In some embodiments, the oxylipin 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). In some embodiments, the endocannabinoid is anandamide or 2-arachidonoylglycerol (2-AG). In some embodiments, a cultured milk product disclosed herein further comprises secretory IgA (sIgA). In some embodiments, the cultured milk product does not comprise or is substantially free of persistent organic pollutants (POPs). In some embodiments, 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. In some embodiments, the cultured milk product does not comprise or is substantially free of DDT. In some embodiments, the cultured milk product does not comprise or is substantially free of heavy metals. In some embodiments, the cultured milk product does not comprise or is substantially free of mercury, lead, arsenic, cadmium, nickel, chromium, cobalt, or zinc. In some embodiments, the cultured milk product does not comprise or is substantially free of pharmaceutical or recreational drugs. In some embodiments, the cultured milk product does not comprise or is substantially free of chemotherapeutics, antidepressants, or anti-anxiety medications. In some embodiments, the cultured milk product does not comprise or is substantially free of benzodiazepines. In some embodiments, the cultured milk product does not comprise or is substantially free of lithium, carbamazepine, chlorpromazine, and clozapine. In some embodiments, the cultured milk product does not comprise or is substantially free of alcohol, cannabis, opioids, phencyclidine, or cocaine. In some embodiments, the cultured milk product does not comprise or is substantially free of allergens. In some embodiments, the cultured milk product does not comprise or is substantially free of ovomucoid, ovalbumin, conalbumin, arachin 6, arachin 3, conarachin, AraH1, and arachin AraH2. In some embodiments, 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). In some embodiments, the cultured milk product does not comprise or is substantially free of myoepithelial cells, myeloid precursor cells, neutrophils, granulocytes, or T cells. In some embodiments, the cultured milk product does not comprise or is substantially free of Staphylococcus, Acinetobacter, Streptococcus, Pseudomonas, Lactococcus, Enterococcus or Lactobacillus. In some embodiments, the cultured milk product does not comprise or is substantially free of virus. In some embodiments, 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. In some embodiments, the cultured milk product does not comprise or is substantially free of hormones. In some embodiments, 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.

[0009] Disclosed herein, in certain embodiments, are cultured milk products, 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 (tridecyl 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-gamma-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); 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 composition 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 composition may comprise human mammary epithelial cells or plasma cells.

[0010] In some embodiments, a cultured milk product disclosed herein further comprises a preservative, stabilizer, antioxidant, emulsifier, or thickener. In some embodiments, a cultured milk product disclosed herein further comprises lecithin. In some embodiments, a cultured milk product disclosed herein further comprises carrageenan. In some embodiments, a cultured milk product disclosed herein further comprises beta carotene. In some embodiments, a cultured milk product disclosed herein further comprises vitamin E. In some embodiments, a cultured milk product disclosed herein further comprises ascorbyl palmitate or ascorbic acid. In some embodiments, a cultured milk product disclosed herein further comprises lecithin, ascorbyl palmitate and vitamin E. In some embodiments, a cultured milk product disclosed herein is dehydrated, freeze-dried, or frozen. In some embodiments, a cultured milk product disclosed herein is food-grade.

[0011] Disclosed herein, in certain embodiments, are methods of feeding a subject in need thereof, comprising administering to the subject a cultured milk product disclosed herein. In some embodiments, the subject is a human. In some embodiments, the human subject is an infant. In some embodiments, the human subject is immuno-compromised. In some embodiments, the human subject has a disease selected from: severe combined immunodeficiency (SCID), HIV/AIDS, a cancer, or an autoimmune disease. In some embodiments, the human subject has lupus or diabetes (for example, Type I diabetes or Type II diabetes). In some embodiments, the human subject is an organ or bone marrow transplant recipient. In some embodiments, the human subject is malnourished. In some embodiments, the human subject has malabsorption syndrome. In some embodiments, the human subject has wasting syndrome. In some embodiments, the human subject is geriatric.

[0012] Disclosed herein, in certain embodiments, are methods of treating or preventing a microbial infection in a subject in need thereof, comprising administering to the subject a cultured milk product disclosed herein. In some embodiments, the infection is a bacterial infection, fungal infection or parasitic infection. In some embodiments, 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*. In some embodiments, the parasitic infection is an infection of *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium* spp., or *Cystoisospora belli*. In some embodiments, the fungal infection is an infection of *Aspergillus*, *Cryptococcus*, or *Pneumocystis jirovecii*. In some embodiments, the microbial infection is a gastrointestinal infection. In some embodiments, the gastrointestinal infection is gastroenteritis. In some embodiments, the microbial infection is Candidiasis.

[0013] Disclosed herein, in certain embodiments, are methods of producing 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 interior 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. In some embodiments, 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. In some embodiments, 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. In some embodiments, the cultured milk product comprises secretory IgA (sIgA). In some embodiments, the bioreactor comprises an apical compartment that is substantially isolated from the interior cavity of the cell construct. In some embodiments, the basal surface of the mammary cells is in fluidic contact with the culture media. In some embodiments, the apical compartment is in fluidic contact with the apical surface of the mammary cells. In some embodiments, the cultured milk product is secreted from the apical surface of the mammary cells into the apical compartment. In some embodiments, the culture media substantially does not contact the cultured milk product. In some embodiments, 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 . In some embodiments, total cell density of plasma cells in the bioreactor is about 200 to 500 plasma

cells per mm². In some embodiments, the culturing is carried out at a temperature of about 27°C to about 39°C. In some embodiments, the culturing is carried out at an atmospheric concentration of CO₂ of about 4% to about 6%.

[0014] Disclosed herein, in certain embodiments, are pharmaceutical compositions comprising (a) sIgA and (b) a pharmaceutically acceptable excipient; wherein the sIgA is manufactured by a method disclosed herein. In some embodiments, the pharmaceutically acceptable excipient is a stabilizer, a surfactant, a buffer or tonicity agent. In some embodiments, 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.

[0015] Disclosed herein, in certain embodiments, are methods 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 disclosed herein. In some embodiments, the pharmaceutically acceptable excipient is a stabilizer, a surfactant, a buffer or tonicity agent. In some embodiments, 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. In some embodiments, the human subject is an infant. In some embodiments, the human subject is immuno-compromised. In some embodiments, the human subject has a disease selected from: severe combined immunodeficiency (SCID), HIV/AIDS, a cancer, or an autoimmune disease. In some embodiments, the subject has lupus or diabetes (for example, Type I diabetes or Type II diabetes). In some embodiments, the human subject is an organ or bone marrow transplant recipient. In some embodiments, the human subject is malnourished. In some embodiments, the human subject has malabsorption syndrome. In some embodiments, the human subject has wasting syndrome. In some embodiments, the human subject is geriatric. In some embodiments, the human subject has cystic fibrosis, COPD, or non-CF bronchiectasis. In some embodiments, the infection is a bacterial infection, fungal infection or parasitic infection. In some embodiments, 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*. In some embodiments, the parasitic infection is an infection of *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium* spp., or *Cystoisospora belli*. In some embodiments, the fungal infection is an infection of *Aspergillus*, *Cryptococcus*, or *Pneumocystis jirovecii*. In some embodiments, the microbial infection is a gastrointestinal infection. In some embodiments, the gastrointestinal infection is gastroenteritis. In some embodiments, the microbial infection is Candidiasis. In some embodiments, the microbial infection is a respiratory infection. In some embodiments, the respiratory infection is pneumonia, bronchitis, Aspergillosis, or

Cryptococcosis. In some embodiments, the respiratory infection is an infection by *B. cepacia*, *P. aeruginosa*, *S. aureus*, *Aspergillus*, *Cryptococcus*, or *Pneumocystis*. In some embodiments, the composition is formulated for inhalation. In some embodiments, the composition is a powder. In some embodiments, the composition is formulation for administration by a nebulizer.

[0016] The present disclosure relates to milk compositions that comprise protein, lipid, and oligosaccharide components and component concentrations that mimic and/or are substantially similar to human breast milk as produced by a lactating female and are produced *in vitro* and/or *ex vivo* from cultured mammary cells.

[0017] Thus, one aspect of the disclosure relates to milk products comprising specified concentrations or amounts by weight of protein components, lipid components human milk oligosaccharide components and lactose, wherein at least one of the protein components, lipid components, HMOs, and lactose is produced by cultured human mammary epithelial cells.

[0018] In an embodiment, disclosed herein are methods 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; (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, mammary progenitor cells, wherein the polarized mammary cells comprise an apical surface and a basal surface; and (b) isolating the cultured milk product. In some embodiments, the cultured milk product comprises secretory IgA (sIgA).

[0019] In some embodiments, the bioreactor comprises an apical compartment that is substantially isolated from the internal cavity of the cell construct. In some embodiments, the basal surface of the mammary cells is in fluidic contact with the culture media. In some embodiments, the apical compartment is in fluidic contact with the apical surface of the mammary cells. In some embodiments, the cultured milk product is secreted from the apical surface of the mammary cells into the apical compartment. In some embodiments, the culture media substantially does not contact the cultured milk product. In some embodiments, total cell density of mammary cells within the bioreactor is at least 10^{11} . In some embodiments, total surface area of mammary cells within the bioreactor is at least 1.5 m^2 . In some embodiments, the matrix material comprises one or more extracellular matrix proteins. In some embodiments, total cell density of plasma cells in the bioreactor is about 200 to 500 plasma cells per mm^2 . In some embodiments, the culturing is carried

out at a temperature of about 27°C to about 39°C. In some embodiments, the culturing is carried out at an atmospheric concentration of CO² of about 4% to about 6%.

[0020] In certain embodiments, described herein are cell constructs, 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; and (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. In some embodiments, the polarized mammary cells comprise an apical surface and a basal surface. In some embodiments, the basal surface of the mammary cells is in fluidic contact with the culture media.

[0021] In some embodiments, 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. In some embodiments, 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. In some embodiments, the mammary cells comprise a constitutively active prolactin receptor protein. In some embodiments, the culture medium comprises a carbon source, a chemical buffering system, one or more essential amino acids, one or more vitamins and/or cofactors, and one or more inorganic salts. In some embodiments, the culture medium further comprises prolactin. In some embodiments, the matrix material comprises one or more extracellular matrix proteins. In some embodiments, the three-dimensional scaffold comprises a natural polymer, a biocompatible synthetic polymer, a synthetic peptide, a composite derived from any of the preceding, or any combination thereof. In some embodiments, the natural polymer is collagen, chitosan, cellulose, agarose, alginate, gelatin, elastin, heparan sulfate, chondroitin sulfate, keratan sulfate, and/or hyaluronic acid. In some embodiments, the biocompatible synthetic polymer is polysulfone, polyvinylidene fluoride, polyethylene co-vinyl acetate, polyvinyl alcohol, sodium polyacrylate, an acrylate polymer, and/or polyethylene glycol.

[0022] In certain aspects, described herein is a milk product comprising: about 6-14 grams per liter (g/L) protein components; about 18-89 g/L lipid components; about 7-14 g/L human milk oligosaccharides (HMOs); and about 64-77 g/L lactose, wherein at least one of the protein components, lipid components, HMOs, and lactose is produced by cultured human mammary epithelial cells.

[0023] In some embodiments, the milk product further comprises one or more immunoglobulins. In some embodiments, the immunoglobulin is secretory IgA.

[0024] In some embodiments, the protein component comprises about 55-65% dry weight of the milk product. In some embodiments, the protein component comprises beta-casein, kappa-casein and alpha-casein. In some embodiments, the beta-casein has a concentration of about 0.5-1.5 g/L, the kappa-casein has a concentration of about 0.5-0.6 g/L, and the alpha-casein has a concentration of about 0.1-0.5 g/L in the milk product. In some embodiments, the beta-casein, kappa-casein and alpha-casein together comprise about 35-45% dry weight percent of the protein component of the milk product. In some embodiments, the beta-casein comprises greater than about 50% of total casein content.

[0025] In some embodiments, the protein component further comprises one or more of alpha-lactalbumin, lysozyme, lactoferrin, haptocorrin, butyrophilin, osteopontin, mucin MC5, mucin BrE3, and lactadherin. In some embodiments, the protein component further comprises serum albumin. In some embodiments, the alpha-lactalbumin has a concentration of about 2.7-3.3 g/L in the milk product. In some embodiments, the lysozyme has a concentration of about 0.2-0.5 g/L in the milk product. In some embodiments, the lactoferrin has a concentration of about 1.0-2.0 g/L in the milk product. In some embodiments, the haptocorrin has a concentration of about 0.07-0.7 g/L in the milk product. In some embodiments, the butyrophilin has a concentration of about 0.03-0.05 g/L in the milk product. In some embodiments, the osteopontin has a concentration of about 0.05-0.2 g/L in the milk product. In some embodiments, the mucin MC5 has a concentration of about 0.5-0.6 g/L in the milk product. In some embodiments, the mucin BrE3 has a concentration of about 0.5-0.7 g/L in the milk product. In some embodiments, the lactadherin has a concentration of about 0.06-0.07 g/L in the milk product. In some embodiments, the serum albumin has a concentration of about 0.025-3.5 g/L, or about 0.01-2 g/L, or about 0.15-1 g/L, or about 0.2-0.7 g/L. In some embodiments, the protein components are of human origin.

[0026] In some embodiments, the lipid component comprises one or more of saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, cholesterol, phospholipids, plasmalogens and sphingolipids. In some embodiments, the saturated fatty acids has a concentration of about 5-34 g/L in the milk product. In some embodiments, the saturated fatty acids comprise palmitic acid, stearic acid, and lauric acid. In some embodiments, the palmitic acid comprises at least about 50% sn-2 form. In some embodiments, palmitic acid has a concentration of about 3-18 g/L in the milk product. In some embodiments, stearic acid has a concentration of about 0.7-5 g/L in the milk product. In some embodiments, lauric acid has a concentration of about 0.5-5 g/L in the milk product. In some embodiments, monounsaturated fatty acids have a concentration of about 7-46 g/L in the milk product. In some embodiments, the monounsaturated fatty acid comprises oleic acid. In some embodiments, the oleic acid comprises at least about 50% sn-1 form. In some embodiments, the oleic acid has a concentration of about 7-45 g/L in the milk product. In some embodiments, polyunsaturated fats have a concentration of about 2-20 g/L in the milk product. In some embodiments, the polyunsaturated fats comprise one or more of linoleic acid, alpha-linolenic acid,

eicosadienoic acid, arachidonic acid, dihomo-gamma-linolenic acid, and docosahexadienoic acid. In some embodiments, linoleic acid has a concentration of about 2-19 g/L in the milk product. In some embodiments, the linoleic acid comprises at least about 50% sn-3 form. In some embodiments, alpha-linolenic acid has a concentration of about 0.5-0.7 g/L in the milk product. In some embodiments, eicosadienoic acid has a concentration of about 0.5-0.7 g/L in the milk product. In some embodiments, arachidonic acid has a concentration of about 0.5-0.7 g/L in the milk product. In some embodiments, dihomo-gamma-linolenic acid has a concentration of about 0.3-0.5 g/L in the milk product. In some embodiments, docosahexadienoic acid has a concentration of about 0.02-0.4 g/L in the milk product. In some embodiments, linoleic acid has a concentration of about 2-19 g/L, alpha-linolenic acid has a concentration of about 0.5-0.7 g/L, eicosadienoic acid has a concentration of about 0.5-0.7 g/L, arachidonic acid has a concentration of about 0.5-0.7 g/L, dihomo-gamma-linolenic acid has a concentration of about 0.3-0.5 g/L, and docosahexadienoic acid has a concentration of about 0.02-0.4 g/L in the milk product. In some embodiments, the milk product comprises oleic acid, palmitic acid, and linoleic acid, wherein the oleic acid comprises at least about 50% sn-1 form, the palmitic acid comprises at least about 50% sn-2 form, and the linoleic acid comprises at least about 50% sn-3 form. In some embodiments, the milk product comprises cholesterol having a concentration of about 0.09-0.15 g/L in milk product. In some embodiments, phospholipids, plasmalogens and sphingolipids together have a concentration of about 0.1-0.4 g/L in the milk product.

[0027] In some embodiments, the human milk oligosaccharide component comprises one or more neutral oligosaccharides. In some embodiments, the human milk oligosaccharide component comprises one or more acidic oligosaccharides. In some embodiments, the human milk oligosaccharide component comprises neutral oligosaccharides and/or acidic oligosaccharides. In some embodiments, the one or more neutral oligosaccharides comprise TF-LNH (trifucosyllacto-N-hexose), 2'-FL (2'-fucosyllactose), DF-LNHII (difucosyllacto-N-hexaose), LNFP I (lacto-N-fucopentaose I), LNDFHI (lacto-N-difucosylhexaose I), LNT (lacto-N-tetraose), LNnT (lacto-N-neotetraose), DF-L (Difucosyllactose), and 3-FL (3-fucosyllactose).

74. The milk product according to claim 65 or 66, wherein the one or more acidic oligosaccharides comprise 6'-SL (6'-sialyllactose), DS-LNT (disialyllacto-N-tetraose), FS-LNnH I (fucosyl-sialyllacto-N-neohexaose I), LST c (sialyl-lacto-N-tetraose c), and 3'-SL (3'-sialyllactose). In some embodiments, the one or more neutral oligosaccharides comprises TF-LNH (trifucosyllacto-N-hexose), which oligosaccharide has a concentration of about 1-4 g/L in the milk product. In some embodiments, the one or more neutral oligosaccharides comprises 2'-FL (2'-fucosyllactose), which oligosaccharide has a concentration of about 1-4 g/L in the milk product. In some embodiments, the one or more neutral oligosaccharides comprises DF-LNHII (difucosyllacto-N-hexaose), which oligosaccharide has a concentration of about 1-4 g/L in the milk product. In some embodiments, the one or more neutral oligosaccharides comprises LNFP I (lacto-N-fucopentaose I), which

oligosaccharide has a concentration of about 0.5-2 g/L in the milk product. In some embodiments, the one or more neutral oligosaccharides comprises LNDFHI (lacto-N-difucosylhexaose I), which oligosaccharide has a concentration of about 0.2-2 g/L in the milk product.

80. The milk product according to any of claims 64, 66, 67, or 70-74, wherein the one or more neutral oligosaccharides comprises LNT (lacto-N-tetraose), which oligosaccharide has a concentration of about 0.3-1.5 g/L in the milk product. In some embodiments, the one or more neutral oligosaccharides comprises LNnT (lacto-N-neotetraose), which oligosaccharide has a concentration of about 0.5-1.5 g/L in the milk product. In some embodiments, the one or more neutral oligosaccharides comprises DF-L (Difucosyllactose), which oligosaccharide has a concentration of about 0.1-1 g/L in the milk product. In some embodiments, the one or more neutral oligosaccharides comprises 3-FL (3-fucosyllactose), which oligosaccharide has a concentration of about 0.2-1.5 g/L in the milk product. In some embodiments, the one or more acidic oligosaccharides comprises 6'-SL (6'-sialyllactose), which oligosaccharide has a concentration of about 0.2-1.2 g/L in the milk product. In some embodiments, the one or more acidic oligosaccharides comprises DS-LNT (disialyllacto-N-tetraose), which oligosaccharide has a concentration of about 0.05-1 g/L in the milk product. In some embodiments, the one or more acidic oligosaccharides comprises FS-LNnH I (fucosyl-sialyl-lacto-N-neohexaose I), which oligosaccharide comprises about 0.05-0.7 g/L in the milk product. In some embodiments, the one or more acidic oligosaccharides comprises LST c (sialyl-lacto-N-tetraose c), which oligosaccharide has a concentration of about 0.05-0.7 g/L in the milk product. In some embodiments, the one or more acidic oligosaccharides comprises 3'-SL (3'-sialyllactose), which oligosaccharide has a concentration of about 0.1-0.3 g/L in the milk product.

[0028] In some embodiments, the one or more neutral oligosaccharides comprise TF-LNH (trifucosyllacto-N-hexose), 2'-FL (2'-fucosyllactose), DF-LNH II (difucosyllacto-N-hexaose), LNFP I (lacto-N-fucopentaose I), LNDFHI (lacto-N-difucosylhexaose I), LNT (lacto-N-tetraose), LNnT (lacto-N-neotetraose), DF-L (Difucosyllactose), and 3-FL (3-fucosyllactose) and wherein the one or more acidic oligosaccharides comprises 6'-SL (6'-sialyllactose), DS-LNT (disialyllacto-N-tetraose), FS-LNnH I (fucosyl-sialyl-lacto-N-neohexaose I), LST c (sialyl-lacto-N-tetraose c), and 3'-SL (3'-sialyllactose). In some embodiments, the milk product comprises about 1-4 g/L TF-LNH (trifucosyllacto-N-hexose), about 1-4 g/L 2'-FL (2'-fucosyllactose), about 1-4 g/L DF-LNH II (difucosyllacto-N-hexaose), about 0.5-2 g/L LNFP I (lacto-N-fucopentaose I), about 0.22 g/L LNDFHI (lacto-N-difucosylhexaose I), about 0.3-1.5 g/L LNT (lacto-N-tetraose), about 0.5-1.5 g/L LNnT (lacto-N-neotetraose), about 0.1-1 g/L DF-L (Difucosyllactose), about 0.2-1.5 g/L 3-FL (3-fucosyllactose), about 0.2-1.2 g/L 6'-SL (6'-sialyllactose), about 0.05-1 g/L DS-LNT (disialyllacto-N-tetraose), about 0.05-0.7 g/L FS-LNnH I (fucosyl-sialyl-lacto-N-neohexaose I), about 0.05-0.7 g/L LST c (sialyl-lacto-N-tetraose c), and about 0.1-0.3 g/L 3'-SL (3'-sialyllactose). In some embodiments, the neutral oligosaccharides content comprises at least about 2-fold, or about 3-fold,

or about 4-fold, or about 5-fold, or about 6-fold, or about 7-fold, or about 8-fold, or about 9-fold, or about 10-fold, or about 11-fold, or about 12-fold, or about 13-fold, or about 14-fold, or about 15-fold more by weight than acidic oligosaccharide content.

[0029] In certain aspects, described herein are milk products comprising: about 6-14 grams per liter (g/L) protein components; about 18-89 g/L lipid components; about 7-14 g/L human milk oligosaccharides (HMOs); and about 64-77 g/L lactose, wherein the protein components comprise beta-casein, kappa-casein, and alpha-casein, alpha-lactalbumin, lysozyme, lactoferrin, haptocorrin, butyrophilin, osteopontin, mucin MC5, mucin BrE3, and lactadherin, wherein the lipid components comprise palmitic acid, stearic acid and lauric acid, oleic acid, linoleic acid, alpha-linolenic acid, eicosadienoic acid, arachidonic acid, dihomo-gamma-linolenic acid, docosahexadienoic acid, cholesterol, phospholipids, plasmalogens and sphingolipids, wherein the human milk oligosaccharides comprise TF-LNH (trifucosyllacto-N-hexose), 2'-FL (2'-fucosyllactose), DF-LNHII (difucosyllacto-N-hexaose), LNFP I (lacto-N-fucopentaose I), LNDFHI (lacto-N-difucosylhexaose I), LNT (lacto-N-tetraose), LNnT (lacto-N-neotetraose), DF-L (Difucosyllactose), and 3-FL (3-fucosyllactose), 6'-SL (6'-sialyllactose), DS-LNT (disialyllacto-N-tetraose), FS-LNnH I (fucosyl-sialyl-lacto-N-neohexaose I), LST c (sialyl-lacto-N-tetraose c), and 3'-SL (3'-sialyllactose), and wherein at least one of the protein components, lipid components, HMOs, and lactose is produced by cultured human mammary epithelial cells.

[0030] In some embodiments, the milk product comprises about 0.5-1.5 g/L beta-casein, about 0.5-0.6 g/L kappa-casein, about 0.1-0.5 g/L alpha-casein, about 2.7-3.3 g/L alpha-lactalbumin, about 0.2-0.5 g/L lysozyme, about 1.0-2.0 g/L lactoferrin, about 0.07-0.7 g/L haptocorrin, about 0.03-0.05 g/L butyrophilin, about 0.05-0.2 g/L osteopontin, about 0.5-0.6 g/L mucin MC5, about 0.5-0.7 g/L mucin BrE3, about 0.06-0.07 g/L lactadherin, about 2-19 g/L linoleic acid, about 0.5-0.7 g/L alpha-linolenic acid, about 0.5-0.7 g/L eicosadienoic acid, about 0.5-0.7 g/L arachidonic acid, about 0.3-0.5 g/L dihomo-gamma-linolenic acid, about 0.02-0.4 g/L docosahexadienoic acid, about 0.09-0.15 g/L cholesterol, about 0.1-0.4 g/L phospholipids, plasmalogens, and sphingolipids combined, about 1-4 g/L TF-LNH (trifucosyllacto-N-hexose), about 1-4 g/L 2'-FL (2'-fucosyllactose), about 1-4 g/L DF-LNHII (difucosyllacto-N-hexaose), about 0.5-2 g/L LNFP I (lacto-N-fucopentaose I), about 0.2-2 g/L LNDFHI (lacto-N-difucosylhexaose I), about 0.3-1.5 g/L LNT (lacto-N-tetraose), about 0.5-1.5 g/L LNnT (lacto-N-neotetraose), about 0.1-1 g/L DF-L (Difucosyllactose), about 0.2-1.5 g/L 3-FL (3-fucosyllactose), about 0.2-1.2 g/L 6'-SL (6'-sialyllactose), about 0.05-1 g/L DS-LNT (disialyllacto-N-tetraose), about 0.05-0.7 g/L FS-LNnH I (fucosyl-sialyl-lacto-N-neohexaose I), about 0.05-0.7 g/L LST c (sialyl-lacto-N-tetraose c), and about 0.1-0.3 g/L 3'-SL (3'-sialyllactose).

[0031] In certain aspects, described herein are milk products comprising: about 3-15 percent protein by weight; about 9-92 percent lipid by weight; about 4-15 percent by weight human milk oligosaccharides (HMOs); and about 33-80 percent by weight lactose, wherein at least one of the

protein, lipid, HMOs, and lactose is produced by cultured human mammary epithelial cells. In some embodiments, the milk product further comprises serum albumin. In some embodiments, the milk product comprises about 0.025-3.5 g/L, or about 0.01-2 g/L, or about 0.15-1 g/L, or about 0.3-0.7 g/L serum albumin.

[0032] In some embodiments, the milk product further comprises one or more immunoglobulins. In some embodiments, the immunoglobulins comprise one or more of IgA, IgG and IgM. In some embodiments, the IgA comprises one or more of IgA2 (secretory) and IgA1 (non-secretory). In some embodiments, the milk product comprises about 0.2-1.0 g/L secretory IgA. In some embodiments, the milk product comprises about 0.15-1.6 g/L total IgA. In some embodiments, the milk product comprises about 0.03-0.3 g/L IgG. In some embodiments, the milk product comprises about 0.01-0.1 g/L IgM. In some embodiments, the milk product comprises about 0.2-2 percent by weight immunoglobulins.

[0033] In some embodiments, the protein components, lipid components, HMOs, and lactose are isolated from cultured mammary epithelial cells. In some embodiments, the cultured mammary epithelial cells comprise one or more immortalized mammary cell lines. In some embodiments, the cultured mammary epithelial cells are derived from one or more primary mammary tissue samples. In some embodiments, the one or more primary mammary tissue samples is derived from a surgical explant of mammary gland tissue. In some embodiments, the one or more primary mammary tissue samples comprises alveolar and/or luminal tissue or cells collected from the alveolar structure of the mammary gland. In some embodiments, the one or more primary mammary tissue samples is derived from a needle aspiration of mammary gland tissue. In some embodiments, the primary mammary tissue further comprises one or more myoepithelial cells. In some embodiments, the primary mammary tissue further comprises one or more stem cells. In some embodiments, the primary mammary tissue further comprises one or more immune cells. In some embodiments, the immune cells comprise B cells. In some embodiments, the immune cells comprise plasma cells.

[0034] In some embodiments, the milk product is isolated from cultured mammary epithelial cells from one or more specific persons. In some embodiments, the milk product isolated from cultured mammary epithelial cells from a specific donor is provided only to that donor or persons authorized by that donor. In some embodiments, the cultured mammary epithelial cells are co-cultured with one or more plasma cells lines. In some embodiments, the cultured mammary epithelial cells are co-cultured with one or more myoepithelial cell lines. In some embodiments, the cultured mammary epithelial cells are co-cultured with one or more stem cell lines.

[0035] In some embodiments, the milk product comprises at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 97%, or at least about 98%, or at least about 99%, of the overall macromolecular composition of human breast milk. In some embodiments,

non-protein nitrogen content comprises at least about 10%, or at least about 15%, or at least about 20%, or at least about 25%, or at least about 30% of total nitrogen content.

[0036] In certain aspects, described herein are frozen milk products, comprising a milk product described herein that has been frozen. In some embodiments, the milk product has been lyophilized. In certain aspects, described herein is a containerized milk product, comprising a milk product described herein that is packaged into a container. In certain aspects, described herein is a containerized frozen milk product, comprising the frozen milk product that is packaged into a container. In certain aspects, described herein is a containerized lyophilized milk product, comprising the lyophilized milk product that is packaged into a container.

[0037] In certain aspects, described herein are extracted milk products, comprising one or more components extracted from a milk product described herein. In some embodiments, the one or more components extracted from the collected milk product are lyophilized or concentrated to produce a lyophilized or a concentrated extracted milk product component. In some embodiments, the one or more components extracted from the collected milk product are concentrated by membrane filtration or reverse osmosis. In some embodiments, the one or more extracted components from the collected milk product comprise milk protein, lipid, carbohydrate, vitamin, and minerals.

[0038] In certain aspects, described herein are containerized extracted milk products, comprising the extracted milk product described herein that is packaged in a container. In some embodiments, the container is sterile. In some embodiments, the container is vacuum-sealed. In some embodiments, the container is a food grade container. In some embodiments, the container is a canister, a jar, a bottle, a bag, a box, or a pouch.

[0039] In some embodiments, the milk product comprises about 500-1150 kcal/L available energy content. In some embodiments, between about 40-55% of the available energy content of the milk product is from lipids. In some embodiments, the milk product comprises between about 95.8 and 195.2 g/L macromolecular content.

[0040] In certain aspects, described herein are methods of treating a patient suffering from an infectious disease, comprising: administering to the patient an effective amount of a milk product comprising: about 6-14 grams per liter (g/L) protein components; about 18-89 g/L lipid components; about 7-14 g/L human milk oligosaccharides (HMOs); and about 64-77 g/L lactose, wherein at least one of the protein components, lipid components, HMOs, and lactose. In some embodiments, the milk product further comprises one or more immunoglobulins. In some embodiments, the immunoglobulins comprise one or more of IgA, IgG and IgM. In some embodiments, the IgA comprises one or more of IgA2 (secretory) and IgA1 (non-secretory).

[0041] In some embodiments, the milk product comprises about 0.2-1.0 g/L secretory IgA. In some embodiments, milk product comprises about 0.15-1.6 g/L total IgA. In some embodiments, the milk

product comprises about 0.03-0.3 g/L IgG. In some embodiments, the milk product comprises about 0.01-0.1 g/L IgM. In some embodiments, the milk product comprises about 0.2-2 percent by weight immunoglobulins. In some embodiments, the infectious disease is a gastrointestinal infection. In some embodiments, the patient is immune compromised.

[0042] In certain aspects, described herein are methods of treating an infant suffering from a gastrointestinal infection comprising administering an effective amount of a milk product comprising: about 6-14 grams per liter (g/L) protein components; about 18-89 g/L lipid components; about 7-14 g/L human milk oligosaccharides (HMOs); and about 64-77 g/L lactose, wherein at least one of the protein components, lipid components, HMOs, and lactose. In some embodiments, the milk product further comprises one or more immunoglobulins. In some embodiments, the immunoglobulins comprise one or more of IgA, IgG and IgM. In some embodiments, the IgA comprises one or more of IgA2 (secretory) and IgA1 (non-secretory). In some embodiments, the milk product comprises about 0.2-1.0 g/L secretory IgA. In some embodiments, the milk product comprises about 0.15-1.6 g/L total IgA. In some embodiments, the milk product comprises about 0.03-0.3 g/L IgG. In some embodiments, the milk product comprises about 0.01-0.1 g/L IgM. In some embodiments, the milk product comprises about 0.2-2 percent by weight immunoglobulins.

[0043] These and other aspects of the disclosure are set forth in more detail in the description of the disclosure below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0044] FIG. 1 shows an example of the collection of milk for nutritional use from mammary epithelial cells grown as a confluent monolayer in a compartmentalizing culture apparatus in which either fresh or recycled media is provided to the basal compartment and milk is collected from the apical compartment. TEER, transepithelial electrical resistance.

[0045] FIG. 2 shows an example of polarized absorption of nutrients and secretion of milk across a confluent monolayer of mammary epithelial cells anchored to a scaffold at the basal surface.

[0046] FIG. 3 shows an example micropatterned scaffold that provides increased surface area for the compartmentalized absorption of nutrients and secretion of milk by a confluent monolayer of mammary epithelial cells.

[0047] FIG. 4 shows three examples of a hollow fiber bioreactor depicted as a bundle of capillary tubes (top), which can support mammary epithelial cells lining either the external (top and lower left) or internal (lower right) surface of the capillaries, providing directional and compartmentalized absorption of nutrients and secretion of milk.

[0048] FIG. 5 exemplifies a cross-section of three-dimensional cell construct. The construct is made up of a scaffold having an interior surface defining an interior cavity/basal chamber and an exterior surface. The interior cavity/basal chamber comprises cell culture media. A matrix material sits on

top of the exterior surface of the scaffold. Pores transverse the scaffold from the interior surface to the exterior surface, allowing cell media to contact the basal surface of the cells of the cell monolayer disposed on the matrix material.

[0049] FIG. 6 exemplifies a bioreactor for producing a cultured milk product. The bioreactor is made up of a cell construct and an apical chamber. The cell construct is made up of a scaffold having an interior surface defining an interior cavity/basal chamber and an exterior surface. The cavity comprises cell culture media. A matrix material sits on top of the exterior surface of the scaffold. Pores transverse the scaffold from the interior surface to the exterior surface, allowing cell media to contact the basal surface of the cells of the cell monolayer disposed on the matrix material. The apical surface of the cells of the cell monolayer secrete the milk/cultured milk product into the apical chamber. The apical chamber and the interior cavity/basal chamber are separated by the cell monolayer.

[0050] FIG. 7 exemplifies a cell construct. The construct is made up of a scaffold having an interior surface defining an interior cavity/basal chamber and an exterior surface. The interior cavity/basal chamber comprises cell.

[0051] FIG. 8 exemplifies a cell construct having mammary epithelial cells (MECs) and plasma cells. The plasma cells are adjacent to the scaffold. The MECs form a confluent monolayer above (and in some instances, in between) the plasma cells, with the apical side of the MECs facing the apical compartment (or, milk compartment). The plasma cells secrete IgA, which then binds to a receptor on the basolateral surface of the MECs, triggering internalization of the antibody-receptor complex and further processing of the antibody into sIgA as it transits toward the apical surface (not shown).

[0052] FIG. 9 Production of cell-cultured human milk. (A) Cultivation of mammary epithelial cells for production of milk in a hollow fiber bioreactor. MECs are expanded in tissue culture flasks (1) and then inoculated into the extracapillary space of a hollow fiber bioreactor (2), where they attach to ECM components that coat the surface of the fibers for further proliferation over the large surface area. Media (3) is circulated through the fibers by a peristaltic pump (4). Cellular metabolism is monitored by daily measurement of glucose utilization (5). When the MECs reach confluence and become contact inhibited, the culture media is switched to a lactogenic formulation containing prolactin, and the product is harvested from the extracapillary space (6). (B) Monitoring mammary epithelial cell growth and milk biosynthesis in a hollow fiber bioreactor. Glucose utilization (solid line) and lactose synthesis (dashed line) were monitored daily after inoculation of human primary mammary epithelial cells into a hollow fiber bioreactor using a diabetic glucose monitor (AccuCheck) and a fluorometric lactose assay (Sigma-Aldrich), respectively. (C) Detection of milk protein synthesis. Samples collected from the extracapillary space were submitted to capillary electrophoresis and immunodetection of human beta-casein. (D) Quantification of total protein in the

apical (extracapillary space) and basal (culture media) compartments of a hollow fiber bioreactor culture of hMECs.

DETAILED DESCRIPTION

[0053] Milk is a nutrient-rich liquid food produced in the mammary glands of mammals. It is a primary source of nutrition for infant mammals (including humans who are breastfed) before they are able to digest other types of food. Milk is a complex biological matrix composed of thousands of unique molecules, with nutritional and functional properties that are ideal to support the growth and development of a mammalian infant.

[0054] Natural milk contains many macronutrients, including proteins, lipids, polysaccharides and lactose. Each species produces milk with a unique composition that reflects its distinct physiological needs (Beck KL, et al., *JProteome Res.* 2015;14(5):2143-2157). As milk composition is optimized for each species, breast milk is considered the gold standard for human infant nutrition and is recommended as the exclusive nutrition source for the first 6 months by the World Health Organization (Mordor Intelligence, *Global dairy market* (2016-2024)).

[0055] Compared with bovine milk, human milk is lower in protein but higher in fat and carbohydrate content (Ballard O, et al., *Pediatr Clin North Am.* 2013;60(1):49-74). Many components overlap but undergo different processing steps in humans and cows and therefore differ in their final conformations and physiological properties. For example, the positions of specific fatty acids in triglycerides differ between bovine milk and breast milk, and these differences have been shown to affect absorption (Andreas NJ, et al., *Early Hum Dev.* ;91 (11):629-635). Additionally, human milk contains hundreds of unique oligosaccharides that contribute to gut maturation and immunity in the developing infant, which are lacking in bovine-based formula (Totten SM, et al., *JProteome Res.* 2012;11(12):6124-6133).

[0056] Human milk is not merely nutritional. Rather, human milk contains a variety of factors with bioactive qualities that have a profound role in infant survival and health. Early milk from mammals contains antibodies that provide protection to the newborn baby. Additional factors contributing to the disease fighting potential of natural milk include leukocytes, hormones, antimicrobial peptides, cytokines, chemokines, and other bioactive factors.

[0057] Secretory IgA (sIgA) is found in naturally-occurring breast milk. IgA is produced by plasma cells located within the breast tissue. The IgA binds to a receptor (polymeric Ig receptor) on the basal surface of mammary epithelial cells. The IgA and receptor are transported into the mammary epithelial cells where the mammary cells process the IgA by cleaving the extracellular domain of the polymeric Ig receptor bound to the IgA, leaving the soluble ectodomain of the Ig receptor (the "secretory component") bound to the IgA. The sIgA is secreted from the apical surface of the mammary epithelial cells. sIgA transferred to children via breast milk is an integral component of an

infant's immune system enabling them to fight off infections. Further, sIgA is an important component of the immune systems of all humans and sIgA deficiencies are associated with increased susceptibility to illness. sIgA-based therapies offer the opportunity to prevent and treat infectious diseases at their sites of entry.

[0058] Immune cells, including CD20+ B cells, which are precursors for sIgA-secreting plasma cells, are present in normal, non-lactating mammary tissue. During lactation, immune cell homing to the mammary gland contributes to a local, mucosal immunity that helps to prevent infection within the mammary gland. Further, immune cell homing to the mammary gland enables the transfer of sIgA antibodies to an infant via breast feeding.

[0059] Unfortunately, efforts at mass production of sIgA are hampered by the fact that it is a multivalent and heavily post-translationally modified protein assembly that requires cooperation with a mucosal epithelium for processing into its bioactive form. Thus, it is difficult to manufacture sIgA for use with non-naturally occurring breast milk or as a therapeutic for humans.

[0060] These are several of the factors that contribute to the inability of infant formula to replicate the properties of breast milk. Therefore, there is a need for improved milk products for developing infants and children that more closely mimic the composition of human breast milk as produced by a lactating female.

[0061] Mammary epithelial cells (MECs) in culture have been previously demonstrated to display organization and behavior similar to that observed *in vivo* (Arevalo et al. 2016 *Am J Physiol Cell Physiol.* 310(5) :C348-3 56; Chen et al. 2019 *Curr Protoc Cell Biol.* 82(1):e65). In Arevalo et al., specific biomarkers of MEC populations were detected in immortalized bovine mammary epithelial cells (BME-UV1) and immortalized bovine mammary alveolar cells (MAC-T) cultured on adherent 2-D plates, ultralow attachment surface 3D microplates, and 3D plates coated with Matrigel. Additionally, in Chen et al., protocols are detailed for isolation and culture of human primary mammary epithelial stem/progenitor cells from human breast tissue and subsequent generation of mammospheres using 3D organoid culture on gelatin sponges and Matrigel matrices. However, neither Arevalo nor Chen attempted to stimulate the production of milk from these MEC cultures.

[0062] In particular, when grown on an appropriate extracellular matrix and stimulated with prolactin, cultured bovine mammary epithelial cells polarize and organize into structures capable of secreting certain milk components (Blatchford et al. 1999 *Animal Cell Technology: Basic & Applied Aspects* 10:141-145). In Blatchford et al, bovine MECs polarized and formed mammospheres. Casein and butyrophilin were isolated from the cultures. However, the cells did not polarize in one uniform direction. Blatchford, et al. noted that the milk proteins were distributed in between the cells and dispersed throughout the mammospheres. Due to the lack of a uniform polarization orientation, Blatchford had to isolate the secreted proteins from the culture media.

[0063] Furthermore, *in vitro* two-dimensional models, such as those used in Blatchford et al. provide a low surface area-to-volume ratio (low density format). The surface area available for cell attachment limits the number of cells that can be grown

[0064] The only known attempt to culture mouse mammary epithelial cells in a high-density format, such as the hollow fiber bioreactor, failed to achieve compartmentalization necessary for the production and extraction of a cultured milk product (Sharfstein et al. 1992 *Biotechnology and Bioengineering* 40:672-680). In Sharfstein et al., growth, long-term expression of functional differentiation, and metabolism of COMMA-1D (an immortalized mouse mammary epithelial cell line) was examined in two different systems: extended batch culture and hollow-fiber reactor culture. Using COMMA-1D seeded onto Costar Transwell® polycarbonate membrane cell culture inserts, Sharfstein et al. created a confluent monolayer capable of barrier formation and polarized metabolism between the apical and basal side that maintained gradients of glucose and lactate. However, using a hollow-fiber bioreactor culture, Sharfstein et al. was unable to achieve separation of basal and apical compartments. Furthermore, it was not determined if nutrient uptake was polarized in a hollow-fiber culture (Sharfstein et al. 1992). Importantly, no prior work has been able to culture mammary epithelial cells from humans or other nutritionally relevant species in a high-density, three-dimensional, compartmentalizing format.

[0065] The present disclosure is explained in greater detail below. This description is not intended to be a detailed catalog of all the different ways in which the disclosure may be implemented, or all the features that may be added to the instant disclosure. For example, features illustrated with respect to one embodiment may be incorporated into other embodiments, and features illustrated with respect to a particular embodiment may be deleted from that embodiment. Numerous variations and additions to the various embodiments suggested herein will be apparent to those skilled in the art in light of the instant disclosure, which do not depart from the instant disclosure. Hence, the following specification is intended to illustrate some particular embodiments of the disclosure, and not to exhaustively specify all permutations, combinations, and variations thereof.

[0066] Unless the context indicates otherwise, it is specifically intended that the various features of the disclosure described herein can be used in any combination. Moreover, the present disclosure also contemplates that in some embodiments of the disclosure, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a complex comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

Definitions

[0067] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure

belongs. The terminology used in the description of the disclosure herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the disclosure.

[0068] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. As used herein, the singular forms “a,” “an,” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. It will be further understood that the terms “comprises” and/or “comprising,” when used in this specification, specify the presence of stated features, elements, and/or components, but do not preclude the presence or addition of one or more other features, elements, components, and/or groups thereof. As used herein, the term “and/or” includes any and all combinations of one or more of the associated listed items. As used herein, phrases such as “between X and Y” and “between about X and Y” should be interpreted to include X and Y. As used herein, phrases such as “between about X and Y” mean “between about X and about Y.” As used herein, phrases such as “from about X to Y” mean “from about X to about Y.”

[0069] Moreover, the present disclosure also contemplates that in some embodiments, any feature or combination of features set forth herein can be excluded or omitted.

[0070] Furthermore, the term “about,” as used herein when referring to a measurable value such as an amount of a compound or agent of this disclosure, dose, time, temperature, and the like, is meant to encompass variations of $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, $\pm 0.5\%$, or even $\pm 0.1\%$ of the specified amount.

[0071] As used herein, the transitional phrase “consisting essentially of” is to be interpreted as encompassing the recited materials or steps and those that do not materially affect the basic and novel characteristic(s) of the disclosure. Thus, the term “consisting essentially of” as used herein should not be interpreted as equivalent to “comprising.”

[0072] As used herein, the compositions described in the present disclosure are referred to interchangeably as (the singular or plural forms of) “nutritional compositions substantially similar to human milk,” “milk products,” “milk compositions,” “cultured milk products,” or equivalent as made clear by the context.

[0073] As used herein, by “isolate” (or grammatical equivalents, e.g., “extract”) a product, it is meant that the product is at least partially separated from at least some of the other components in the starting material.

[0074] As used herein, the term “polypeptide” encompasses both peptides and proteins, and does not require any particular amino acid length or tertiary structure unless indicated otherwise.

[0075] The term “cultured milk product” and “milk product” are used interchangeably to mean the product secreted by the apical surface of a live cell construct (or, cell culture) comprising human mammary epithelial cells (hMEC). In some embodiments, the live cell construct is cultured in a bioreactor.

[0076] The term “polarized” as used herein in reference to cells and/or monolayers of cells refers to a spatial status of the cell wherein there are two distinct surfaces of the cell, e.g., an apical surface and a basal surface, which may be different. In some embodiments, the distinct surfaces of a polarized cell comprise different surface and/or transmembrane receptors and/or other structures. In some embodiments, individual polarized cells in a continuous monolayer have similarly oriented apical surfaces and basal surfaces. In some embodiments, individual polarized cells in a continuous monolayer have communicative structures between individual cells (e.g., tight junctions) to allow cross communication between individual cells and to create separation (e.g., compartmentalization) of the apical compartment and basal compartment.

[0077] As used herein, “apical surface” means the surface of a cell that faces an external environment or toward a cavity or chamber, for example the cavity of an internal organ. With respect to mammary epithelial cells, the apical surface is the surface from which the cultured milk product is secreted.

[0078] As used herein, “basal surface” means the surface of a cell that is in contact with a surface, e.g., the matrix of a bioreactor.

[0079] As used herein, “bioreactor” means a device or system that supports a biologically active environment that enables the production of a cultured milk product described herein from mammary cells described herein.

[0080] The term “lactogenic” as used herein refers to the ability to stimulate production and/or secretion of milk. A gene or protein (e.g., prolactin) may be lactogenic, as may any other natural and/or synthetic product. In some embodiments, a lactogenic culture medium comprises prolactin, thereby stimulating production of milk by cells in contact with the culture medium.

[0081] As used herein, the term “food grade” refers to materials considered non-toxic and safe for consumption (e.g., human and/or other animal consumption), e.g., as regulated by standards set by the U.S. Food and Drug Administration.

Cell Constructs

[0082] Included herein are cell constructs for producing a cultured milk product representing the biosynthetic output of cultured mammary epithelial cells (immortalized or from primary tissue samples) and IgA producing cells, for example plasma cells. Disclosed herein, in certain embodiments, are cell constructs for producing cultured milk products comprising sIgA, the cell constructs 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 population of plasma cells (PCs) disposed on the

matrix material and (e) a continuous monolayer of mammary cells disposed on the population of plasma cells, the mammary cells selected from the group consisting of: (i) mammary epithelial cells, (ii) mammary myoepithelial cells, and (iii) mammary progenitor cells.

Mammary Cells

[0083] In some embodiments, the mammary cells comprise milk-producing mammary epithelial cells (MECs), contractile myoepithelial cells, and/or progenitor cells that can give rise to both mammary epithelial cells (MECs) and mammary contractile myoepithelial cells. Mammary epithelial cells (MECs) are the only cells that produce milk. In some embodiments, the mammary cells comprise mammary epithelial cells (MECs), primary mammary epithelial cells, mammary myoepithelial cells and mammary progenitor cells. In some embodiments, the mammary cells are obtained from a tissue biopsy of a mammary gland.

[0084] In some embodiments, the mammary cells are derived from breast milk-derived stem cells or breast stem cells originating from tissue biopsy of a mammary gland. The epithelial component of breast milk includes not only mature epithelial cells, but also their precursors and stem cells in culture. A subpopulation of breast milk-derived stem cells displays very high multilineage potential, resembling those typical for human embryonic stem cells (hESCs). Breast stem cells may also originate from tissue biopsy of the mammary gland, and include terminally differentiated MECs. Both breast milk-derived stem cells and breast stem cells originating from tissue biopsy of the mammary gland are multi-potent cells that can give rise to MECs or myoepithelial cells.

[0085] In some embodiments, at least 50% of the mammary cells of the cells culture are polarized. In some embodiments, at least 55% of the mammary cells of the cell culture are polarized. In some embodiments, at least 60% of the mammary cells of the cell culture are polarized. In some embodiments, at least 65% of the mammary cells of the cell culture are polarized. In some embodiments, at least 70% of the mammary cells of the cell culture are polarized. In some embodiments, at least 75% of the mammary cells of the cell culture are polarized. In some embodiments, at least 80% of the mammary cells of the cell culture are polarized. In some embodiments, at least 85% of the mammary cells of the cell culture are polarized. In some embodiments, at least 90% of the mammary cells of the cell culture are polarized. In some embodiments, at least 95% of the mammary cells of the cell culture are polarized. In some embodiments, substantially all of the mammary cells of the cell construct are polarized (i.e., have an apical surface and a basal surface). In some embodiments, substantially all the mammary cells of the cell construct are polarized and substantially all the polarized cells are oriented in the same direction. For example, in some embodiments, substantially all of the mammary cells have an apical surface and a basal surface, wherein the apical surface of substantially all of the cells is oriented in the same direction and the basal surface of substantially all of the cells is oriented in the same direction.

[0086] In some embodiments, the continuous monolayer of mammary cells has at least 70% confluence over the scaffold. In some embodiments, the continuous monolayer of mammary cells has at least about 75% confluence over the scaffold. In some embodiments, the continuous monolayer of mammary cells has at least about 80% confluence over the scaffold. In some embodiments, the continuous monolayer of mammary cells has at least about 85% confluence over the scaffold. In some embodiments, the continuous monolayer of mammary cells has at least about 90% confluence over the scaffold. In some embodiments, the continuous monolayer of mammary cells has at least about 95% confluence over the scaffold. In some embodiments, the continuous monolayer of mammary cells has at least about 99% confluence over the scaffold. In some embodiments, the continuous monolayer of mammary cells has 100% confluence over the scaffold.

Genetic Modifications to Mammary Cells

[0087] In some embodiments, the mammary cells comprise a constitutively active prolactin receptor protein. In some embodiments, the mammary cells comprise a constitutively active human prolactin receptor protein. Where the primary mammary epithelial cell or immortalized mammary epithelial cells comprise a constitutively active prolactin receptor, the culture medium does not contain prolactin.

[0088] In some embodiments, the constitutively active human prolactin receptor protein comprises a deletion of amino acids 9 through 187, wherein the numbering is based on the reference amino acid sequence of a human prolactin receptor identified as SEQ ID NO: 1.

[0089] **SEQ ID NO: 1: Human prolactin receptor (GenBank accession number AAD32032.1)**

[0090] MKENVASATVFTLLFLNTCLLNGQLPPGKPEIFKCRSPNKETFTCWWRPGTDGGLPTNYS
 LTYHREGETLMHECPDYITGGPNSCHFGKQYTSMWRTYIMMVNATNQMGSSFSDEL
 YVDVTYIVQPDPPLELAVEVKQPEDRKPYLWIKWSPPTLIDLKTGWFTLLYEIRLKPEKAAE
 WEIHFAGQQTEFKILSLHPGQKYLQVRCKPDHGYWSAWSPATFIQPSDFTMNDTTVWIS
 VAVLSAVICLIHVWAVALKGYSMVTCIFPPVPGPKIKGFD AHLLEK GKSEELLSALGCQDFPP
 TSDYEDLLVEYLEVDDSEDQHLMSVHSKEHPSQGMKPTYLDPD TDSGRGSCDSPSLLSEKC
 EEPQANPSTFYDPEVIEKPENPETHTHTWDPQCISMEGKIPYFHAGGSKCSTWPLPQPSQHNPR
 SSYHNITDVCELAVGPAGAPATLLNEAGKDALKSSQTIKSREEGKATQQREVESFHSETDQD
 TPWLLPQEKTPFGSAKPLDYVEIHKVNKD GALSLLPKQRENSGPKPKPGTPENNKEYAKVS
 GVMDNNILVLVPDPHAKNVACFEESAKEAPPSLEQNQA EKALANFTATSSKRLQLGGLDY
 LDPACFTHSFH

[0091] In some embodiments, the constitutively active human prolactin receptor protein comprising a deletion of the following amino acids:

VFTLLFLNTCLLNGQLPPGKPEIFKCRSPNKETFTCWWRPGTDGGLPTNYS
 LTYHREGETLMHECPDYITGGPNSCHFGKQYTSMWRTYIMMVNATNQMGSSFSDEL
 YVDVTYIVQPDPPLELAVEVKQPEDRKPYLWIKWSPPTLIDLKTGWFTLLYEIRLKPEKAAE

ELAVEVKQPEDRKPYLWIKWSPPTLIDLKTGWFTLLYEIRLKPEKAA (e.g., amino acid positions 10 through 178 of SEQ ID NO: 1).

[0092] In some embodiments, the mammary cells comprise a loss of function mutation introduced into a circadian related gene *PER2*. In some embodiments, the loss of function mutation introduced into a circadian related gene *PER2* promotes increased synthesis of cultured milk components. In some embodiments, the loss of function mutation in the *PER2* gene comprises an 87-amino acid deletion from position 348 to 434 in *PER2*, wherein the numbering is based on the reference amino acid sequence of a human *PER2* identified as SEQ ID NO: 2.

[0093] **SEQ ID NO: 2: Human Period circadian protein homolog 2 (GenBank accession number NM 022817)**

[0094] MNGYAEFPPSPSNPTKEPVEPQPSQVPLQEDVDMSSGSSGHETNENCSTGRDSQGSD
CDDSGKJELGMLVEPPDARQSPDTFSLMMAKSEHNPSTSGC SSDQSSKVDTHKELIKTLKEL
KVHLPADKKAKGKASTLATLKYALRSVKQVKANEEYYQLMSSEGHPCGADVPSYTVEE
MESVTSEHIVKNADMFAVAVSLVSGKILYISDQVASIFHCKRDAFSDAKFVEFLAPHDVGVF
HSFTSPYKLPLWSMCSGADSFTQECMEEKSFRCRVSVRKSHENEIRYHPFRMTPYL VKVRD
QQGAESQLCCLLLAERVHSGYEAPRIPPEKRIFTTTHTPNCLFQDVDERAVPLLGYLPQD
LIETPVLVQLHPSDRPLMLAIHKKILQSGGQPFDYSPIRFRARNGEYITLDTSWSSFINP
WSRKISFIIGRHKVRVGPLNEDVFAAHPCTEEKALHPSIQELTEQIHRLLLQPVPHSGSSG
YGLSGNSGHEHLMSTSSSDSNGHEDSRRRAEICKNGNKTNRSHYSHESGEQKKKSVT
EMQTNPPAEKKA VPAMEKDSLGVSFPEELACKNQPTCSYQQISCLDSVIRYLESCNEAATLK
RKCEFPANVPALRSSDKRKATVSPGPHAGEAEPPSRVNSRTGVGTHLTSLALPGKAESVASL
TSQCSYSSTIVHVGDKKPQPELEMVEDAASGPESLDCLAGPALACGLS QEKEPFKKLGLTKE
VLA AHTQKEEQSFLQKFKEIRKLSIFQSHCHYYLQERSKGQPSERTAPGLRNTSGIDSPWKK
TGKNRKLKSKRVKPRDSSESTGSGGPVSARPLVGLNATAWSPSDTSQSSCPAVPFPAPVPA
AYSLPVFPAPGTVAAPPAPPHASFTVPAVPVDLQHQAFAVQPPFPAPLAPVMAFMLPSYSFP
SGTPNLPAFFPSQPQFP SHPTLTSEMASASQPEFPEGGTGAMGTTGATETA AVGADCKPGT
SRDQQPKAPLTRDEPSDTQNSDALSTSSGLLNLNLLNEDLCSASGSAASESLGSGSLGCDASPS
GAGSSDTSHTSKYFGSIDSENHAKAMNTGMESEHFIKCVLQDPIWLLMADADSSVMM
TYQLPSRNLEAVLKEDREKLKLLQKLQPRFTESQKQELREVHQWMQTGGLPAAIDVAECV
YCENKEKGNICIPYEEDIPSLGLSEVSDTKEDENGSPLNHRIEEQT

[0095] In some embodiments, the loss of function mutation introduced into *PER2* comprises a deletion of the following amino acids:

CLFQDVDERAVPLLGYLPQDLIETPVLVQLHPSDRPLMLAIHKKILQSGGQPFDYSPIRFRAR
NGEYITLDTSWSSFINPWSRKISFIIGRHKV (e.g., amino acid positions 341 through 434 of SEQ
ID NO:2).

[0096] In some embodiments, the mammary cells comprise a polynucleotide encoding a prolactin receptor comprising a modified intracellular signaling domain. In some embodiments, the loss of function mutation introduced into a circadian related gene *PER2* promotes increased synthesis of individual cultured milk components. In some embodiments, the prolactin receptor comprises a truncation wherein position 154 of exon 10 has been spliced to the 3' sequence of exon 11. In some embodiments, the prolactin receptor comprises a sequence according to SEQ ID NO: 3.

[0097] **SEQ ID NO: 3: Human isoform 4 of Prolactin receptor (GenBank accession number AF416619; Trott et al. 2003 *J. Mol. Endocrinol* 30(1):31-47)**

[0098] MKENVASATVFTLLLFLNTCLLNGQLPPGKPEIFKCRSPNKETFTCWWRPGTDGGLP
TNYSLTYHREGETLMHECPDYITGGPNSCHFGKQYTSMWRTYIMMVNATNQMGSSFSDEL
YVDVTYIVQPDPPLELAVEVKQPEDRKPYLWIKWSPPTLIDLKTGWFTLLYEIRLKPEKAAE
WEIHFAQQQTEFKILSLHPGQKYL VQVRCKPDHGYWSAWSPATFIQPSDFTMNDTTVWIS
VAVLSAVICLIIVWAVALKGYSMVTCIFPPVPGPKIKGFD AHLLEK GKSEELLSALGCQDFPP
TSDYEDLLVEYLEVDDSEDQHLMSVHSKEHPSQGDPLMLGASHYKNLKSYP RKISSQGRL
AVFTKATLTTVQ

[0099] In some embodiments, the mammary cells comprise a polynucleotide encoding a modified (e.g., recombinant) effector of a prolactin protein. In some embodiments, the modified effector of the prolactin protein comprises a janus kinase-2 (JAK2) tyrosine kinase domain. In some embodiments, the modified effector comprises a JAK2 tyrosine kinase domain fused to a signal transducer and activator of transcription-5 (STAT5) tyrosine kinase domain (e.g., a polynucleotide encoding a JAK2 tyrosine kinase domain linked to the 3' end of a polynucleotide encoding the STAT5 tyrosine kinase domain). In some embodiments, the modified effector of a prolactin protein promotes increased synthesis of individual cultured milk components. In some embodiments, the modified effector has a sequence according to SEQ ID NO: 4. Bolded amino acids correspond to the JAK2 kinase domain of amino acid positions 757 through 1129 of a reference human JAK2 amino acid sequence.

[0100] **SEQ ID NO: 4. STA5A Human signal transducer and activator of transcription 5A fused at 3' end to amino acids 757-1129 of JAK2 human tyrosine-protein kinase**

[0101] MAGWIAQQL QGDALRQMQV LYGQHFPIEV RHYLAQWIES QPWDAIDLND
PQDRAQATQL LEGLVQELQK KAEHQVGEDG FLLKIKLGHY ATQLQKTYDR
CPLELVRCIR HILYNEQRLV REANNCSSPA GILVDAMSQK HLQINQTFEE LRLVTQDTEN
ELKKLQQTQE YFIIQYQESL RIQAQFAQLA QLSPQERLSR ETALQQKQVS LEAWLQREAO
TLQQYRVELA EKHQKTLQLL RKQQTHLDD ELIQWKRRQQ LAGNGGPPEG
SLDVLQSWCE KLAELIHWQNR QQIRRAEHL CQQLPIPGPVE EMLAEVNATI TDIISALVTS
TFHIEKQPPQ VLKTQTKFAA TVRLLVGGKL NVHMNPPQVK ATIISEQQAK SLLKNENTRN
ECSGEILNNC CVM EYHQATG TLSAHFRNMS LKRIKRADRR GAESVTEEFK TVLFESQFSV

GSNELVFQVK TSLPWVIV HGSQDHNATA TVLWDNAFAE PGRVPFAVPD
 KVLWPQLCEA LNMKFKAQEVQ SNRGLTKENL VFLAQKLFNN SSSHLEDYSG
 LSVSWSQFNR ENLPGWNYTF WQWFDGVM EV LKKHHKPHWN DGAILGFVNK
 QQAHDLLINK PDGTFLLRFS DSEIGGITIA WKFDSPERNL WNLKPFTTRD FSIRSLADRL
 GDLSYLIYVF PDRPKDEVFS KYYTPVLAKA VDG YVKPQIK QWPEFVNAS ADAGGSSATY
 MDQAPSPAVC PQAPYNMYPQ NPDHVLDDQDG EFDLDETMDV ARHVEELLRR
 PMDSLDSRLS PPAGLFTSAR GSLSLDSQ RKLQFYEDRH QLPAPKWAEL ANLINNCMDY
 EPDFRPSFRA IIRDLNSLFT PDYELLTEND MLPNMRIGAL GFSGAFEDRD PTQFEERHLK
 FLQQLGKGNF GSVEMCRYDP LQDNTGEWA VKKLQHSTEE HLRDFEREIE ILKSLQHDNI
 VKYKGV CYSA GRRNLKLIME YLPYGLRDY LQKHKERIDH IKLLQYTSQI
 CKGMEYLGTK RYIHRDLATR NILVENENRV KIGDFGLTKV LPQDKEYYKV
 KEPGESPIFW YAPESLTESK FSVASDVWSF GWLYELFTY IEKSKSPPAE FMRMIGNDKQ
 GQMIVFHLIE LLKNNGR LPR PDGCPDEIYM IMTECWNNNV NQRPSFRDLA LRVDQIRDN.

[0102] In some embodiments, the mammary cells are immortalized. In some embodiments, the mammary cells comprise one or more nucleic acids encoding human telomerase reverse transcriptase (hTERT) or simian virus 40 (SV40). In some embodiments, the mammary cells comprise a small hairpin RNA (shRNA) to p16 (Inhibitor of Cyclin-Dependent Kinase 4) (p16(INK4)) and Master Regulator of Cell Cycle Entry and Proliferative Metabolism (c-MYC).

[0103] In some embodiments, the method comprises introducing into the mammary cell: (a) a polynucleotide encoding a prolactin receptor comprising a modified intracellular signaling domain, optionally wherein the prolactin receptor comprises a truncation wherein position 154 of exon 10 has been spliced to the 3' sequence of exon 11; (b) a polynucleotide encoding a chimeric prolactin receptor that binds to a ligand, which is capable of activating milk synthesis in the absence of prolactin; (c) a polynucleotide encoding a constitutively or conditionally active prolactin receptor protein, optionally wherein the polynucleotide encodes a constitutively active human prolactin receptor protein comprising a deletion of amino acids 9 through 187 (e.g., a deletion of amino acids 9 through 187, wherein the numbering is based on the reference amino acid sequence of a human prolactin receptor identified as SEQ ID NO: 1); (d) a polynucleotide encoding a modified (e.g., recombinant) effector of a prolactin protein comprising (i) a janus kinase-2 (JAK2) tyrosine kinase domain, optionally wherein the JAK2 tyrosine kinase domain is fused to a signal transducer and activator of transcription-5 (STAT5) tyrosine kinase domain (e.g., a polynucleotide encoding a JAK2 tyrosine kinase domain linked to the 3' end of a polynucleotide encoding the STAT5 tyrosine kinase domain); and/or (ii) a prolactin receptor intracellular domain fused to a JAK2 tyrosine kinase domain; (e) a loss of function mutation into a circadian related gene *PER2* (period circadian protein homolog 2); and/or (f) a polynucleotide encoding one or more glucose transporter genes GLUT1 and/or GLUT12, thereby increasing the rate of nutrient uptake at the basal surface of the monolayer.

Plasma Cells

[0104] Plasma cells are derived from a human donor. In some embodiments, the plasma cells are derived from bone marrow, spleen, and/or a lymph node. a primary mammary tissue sample. In certain embodiments, the plasma cells are derived from mucosal epithelial cells other than mammary cells (e.g., from oronasal, gastrointestinal, or respiratory tissue). In some embodiments, the plasma cells are derived from a plasma cell line. In certain embodiments, the plasma cells are derived from a plasmacyte cell line. In some embodiments, the plasma cells are isolated and sorted from non-plasma cells via fluorescence-activated cell sorting, magnetic-activated cell sorting, and/or microfluidic cell sorting. In some embodiments, plasma cells, plasmablasts, or pre-plasmablasts are sorted and isolated by FACS analysis using markers known in the art (e.g., CD38, CD138 and/or CD19). In certain embodiments, the plasma cells are cultivated with the immortalized mammary epithelial cells on a scaffold, thereby producing a cell construct for producing a cultured milk product with secretory products of the plasma cells and mammary cells (e.g., sIgA). In certain embodiments, the plasma cells are grown on a scaffold below a monolayer of mammary cells. In certain embodiments, the plasma cells are grown as dispersed populations of plasma cells overlaid by a monolayer of mammary cells. In certain embodiments, the plasma cells are stimulated to produce immunoglobins during co-culture with mammary cells. In certain embodiments, the plasma cells produce one or more immunoglobins of a class selected from IgG, IgM and IgA. In certain embodiments the plasma cells produce IgA. In certain embodiments, plasma cells produce IgA, and the IgA is processed by mammary epithelial cells to yield sIgA that is bound to secretory component, and the sIgA is secreted by the apical surface of the mammary cells.

Scaffolds

[0105] In some embodiments, the cell construct further comprises a scaffold having a top surface/exterior surface and a bottom surface/interior surface. In some embodiments, the scaffold is a 2-dimensional surface or a 3-dimensional surface (e.g., a 3-dimensional micropatterned surface, and/or as a cylindrical structure that is assembled into bundles). A non-limiting example of a 2-dimensional surface scaffold is a Transwell® filter. In some embodiments, the scaffold is a 3-dimensional surface. Non-limiting examples of a 3-dimensional micropatterned surface include a microstructured bioreactor, a decellularized tissue (e.g., a decellularized mammary gland or decellularized plant tissue), micropatterned scaffolds fabricated through casting or three-dimensional printing with biological or biocompatible materials, textured surface. In some embodiments, the scaffold is produced by electrospinning cellulose nanofibers and/or a cylindrical structure that can be assembled into bundles (e.g., a hollow fiber bioreactor). In some embodiments, the scaffold is porous. In some embodiments, the scaffold is a 3D scaffold. In some embodiments, the 3-dimensional scaffold is any structure which has an enclosed hollow interior/central cavity. In some embodiments, the three-dimensional scaffold joins with one or more surfaces to form an enclosed

interior chamber/basal compartment. For example, the scaffold can join with one or more walls of a bioreactor to form the interior chamber/basal compartment. In some embodiments, the scaffold is a hollow fiber bioreactor. In some embodiments, the 3D scaffold is a tube in which the central cavity is defined by the interior surface of the scaffold. In some embodiments, the 3D scaffold is a hollow sphere in which the central cavity is defined by the interior surface of the scaffold.

[0106] For *in vitro* culture methods for studies of intestinal absorption, 2-dimensional surface scaffold such as Transwells® have long been used as the standard as they provide both apical and basolateral spaces to simulate the gut-blood-barrier and enable both active and passive transport of drugs and nutrients. However, cells seeded onto flat supports exhibit markedly different phenotypes to cells *in vivo*, partly due to the poor representation of the 3-D extracellular microenvironments.

[0107] A 3-dimensional scaffold allows the cells (e.g., MECs and plasma cells) to grow or interact with their surroundings in all three dimensions. Unlike 2D environments, a 3D cell culture allows cells *in vitro* to grow in all directions, approximating the *in vivo* mammary environment. Further, the 3D scaffold allows for a larger surface area for culture of the cells and for metabolite and gas exchange, plus it enables necessary compartmentalization – enabling the cultured milk product to be secreted into one compartment, while the cell culture media is contacted with the mammary cells and plasma cells in another compartment. To date, a confluent monolayer with polarized separation of basal and apical cell surfaces using mammary epithelial cell on a 3D surface has not been achieved (Sharfstein et al. 1992).

[0108] In some embodiments, the scaffold is porous. In some embodiments, the scaffold is permeable to the cell media, allowing the cell media to contact the cells of the cell monolayer. In some embodiments, the scaffold is transversely by at least one pore that allows the cell media to contact the basal surface of the cells of the cell monolayer.

[0109] In some embodiments, the top surface/exterior surface of the scaffold is coated with a matrix material. In some embodiments, the matrix is made up of one or more extracellular matrix proteins. Non-limiting examples of extracellular matrix proteins include collagen, laminin, entactin, tenascin, and/or fibronectin. In some embodiments, the scaffold comprises a natural polymer, a biocompatible synthetic polymer, a synthetic peptide, and/or a composite derived from any combination thereof. In some embodiments, a natural polymer useful with this invention includes, but is not limited to, collagen, chitosan, cellulose, agarose, alginate, gelatin, elastin, heparan sulfate, chondroitin sulfate, keratan sulfate, and/or hyaluronic acid. In some embodiments, a biocompatible synthetic polymer useful with this invention includes, but is not limited to, cellulose, polysulfone, polyvinylidene fluoride, polyethylene co-vinyl acetate, polyvinyl alcohol, sodium polyacrylate, an acrylate polymer, and/or polyethylene glycol. In some embodiments, the top of the scaffold is coated with laminin and collagen.

[0110] In some embodiments, the matrix material is porous. In some embodiments, the matrix material is permeable to the cell media, allowing the cell media to contact the cells of the cell monolayer. In some embodiments, the matrix material is transversed by at least one pore that allows the cell media to contact the basal surface of the cells of the cell monolayer.

[illegible]

[0112] In some embodiments, the cell construct comprises: (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 population of plasma cells (PCs) disposed on the matrix material and (e) a continuous monolayer of mammary cells disposed on the population of plasma cells, the mammary cells selected from the group consisting of: (i) mammary epithelial cells, (ii) mammary myoepithelial cells, and (iii) mammary progenitor cells; wherein the continuous monolayer mammary epithelial cells has an apical surface and a basal surface (e.g., the cells form a polarized and confluent cell monolayer).

Bioreactor

[0113] Disclosed herein, in certain embodiments, are bioreactors, comprising: (a) an apical compartment comprising a cultured milk product; and (b) at least one 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 population of plasma cells (PCs) disposed on the matrix material and (e) a continuous monolayer of mammary cells disposed on the population of plasma cells, the mammary cells selected from the group consisting of: (i) mammary epithelial cells, (ii) mammary myoepithelial cells, and (iii) mammary progenitor cells. In certain embodiments, the cell construct of the bioreactor comprises at least a 70% confluent monolayer of polarized mammary cells disposed on the matrix material, wherein the mammary cells are selected from the group consisting of: mammary epithelial cells, mammary myoepithelial cells, and mammary progenitor cells; wherein the apical surface of the mammary cells is in fluidic contact with the apical compartment.

[0114] In some embodiments, the bioreactor is an enclosed bioreactor. In some embodiments, the apical chamber is substantially isolated from the interior cavity/basal compartment.

[0115] A hollow fiber bioreactor is an exemplary bioreactor for use with the methods disclosed here. The hollow fiber bioreactor is a high-density, continuous perfusion culture system that closely approximates the environment in which cells grow *in vivo*. It consists of thousands of semi-permeable 3D scaffolds (i.e., hollow fibers) in a parallel array within a cartridge shell fitted with inlet and outlet ports. These fiber bundles are potted or sealed at each end so that any liquid entering the ends of the cartridge will necessarily flow through the interior of the fibers. Cells are generally seeded outside the fibers within the cartridge in the extra capillary space (ECS).

[0116] Three fundamental characteristics differentiate hollow fiber cell culture from other methods: (1) cells are bound to a porous matrix much as they are *in vivo*, not a plastic dish, microcarrier or

other impermeable support, (2) the molecular weight cut off of the support matrix can be controlled, and (3) extremely high surface area to volume ratio (150 cm² or more per mL) which provides a large area for metabolite and gas exchange for efficient growth of host cells.

[0117] The bioreactor structure provides a fiber matrix that allows permeation of nutrients, gases and other basic media components, as well as cell waste products, but not cells, where the cells can be amplified. Hollow fiber bioreactor technology has been used to obtain high density cell amplification by utilizing hollow fibers to create a semi-permeable barrier between the cell growth chamber and the medium flow. Since the surface area provided by this design is large, using this fiber as a culture substrate allows the production of large numbers of cells. Cells growing in the 3-dimensional environment within the bioreactor are bathed in fresh medium as it perfuses through the hollow fibers.

[0118] To replicate the topography of the intestine, Costello et al. developed a 3-D printed bioreactor that can both contain porous villus scaffolds via micromolding (Costello et al. 2017 Scientific Reports 7(12515): 1-10). This geometrically complex molded scaffold provided separation of the apical and basolateral spaces in a manner in which fluid flow exposes intestinal epithelial cells to physiologically relevant shear stresses (Costello et al. 2017). Similarly, a long-term culture in vitro culture in a simulated gut-like environment was created by Morada et al. using a hollow fiber bioreactor which allowed for two controlled separate environments (biphasic) to provide host cells with oxygen and nutrients from the basal layer, while allowing a low oxygen nutrient rich environment to be developed on the apical surface (Morada et al. 2016 International Journal for Parasitology 26: 21-29).

[0119] In configuring the hollow fiber bioreactor, there are design considerations and parameters that can be varied depending upon the goals associated with expansion of the cells. One such design consideration is the size of the pores in the fiber wall. This is generally designed to allow the passage of nutrients to the cells, carry away waste, provide desired products to the cells (such as growth factors), to remove desired products from the cells, and exclude certain factors that may be present from reaching the cells. Accordingly, the pore size of the fiber walls can be varied to modify which components will pass through the walls. For example, pore size can allow the passage of large proteinaceous molecules, including growth factors, including, but not limited to, epidermal growth factor and platelet-derived growth factor. The person of ordinary skill in the art would understand how to vary the pore size depending upon the components that it is desirable to pass through the fiber walls to reach the cells or to carry material from the cells.

[0120] In some embodiments, the pore size is about 0.2 μm. In some embodiments, the pore size is about 0.1 μm. In some embodiments, the pore size is about 0.2 μm. In some embodiments, the pore size is about 0.3 μm. In some embodiments, the pore size is about 0.4 μm. In some embodiments, the pore size is about 0.5 μm. In some embodiments, the pore size is about 0.6 μm. In some

embodiments, the pore size is about 0.7 μm . In some embodiments, the pore size is about 0.8 μm . In some embodiments, the pore size is about 0.9 μm . In some embodiments, the pore size is about 1.0 μm . In some embodiments, the pore size is about 1.1 μm . In some embodiments, the pore size is about 1.2 μm . In some embodiments, the pore size is about 1.3 μm . In some embodiments, the pore size is about 1.4 μm . In some embodiments, the pore size is about 1.5 μm . In some embodiments, the pore size is about 1.6 μm . In some embodiments, the pore size is about 1.7 μm . In some embodiments, the pore size is about 1.8 μm . In some embodiments, the pore size is about 1.9 μm . In some embodiments, the pore size is about 2.0 μm . In some embodiments, the pore size is about 2.1 μm . In some embodiments, the pore size is about 2.2 μm . In some embodiments, the pore size is about 2.3 μm . In some embodiments, the pore size is about 2.4 μm . In some embodiments, the pore size is about 2.5 μm . In some embodiments, the pore size is about 2.6 μm . In some embodiments, the pore size is about 2.7 μm . In some embodiments, the pore size is about 2.8 μm . In some embodiments, the pore size is about 2.9 μm . In some embodiments, the pore size is about 3.0 μm .

Methods of Making Cell Constructs

[0121] Disclosed herein, in certain embodiments, are methods of making a cell construct for producing a cultured milk product comprising immunoglobulins. In some embodiments, the method comprises (a) depositing (i) isolated mammary epithelial cells, mammary myoepithelial cells and/or mammary progenitor cells, and (ii) isolated plasma cells on the upper surface of a scaffold having an upper surface and lower surface to produce a mixed population of plasma cells and mammary cells (i.e., mammary epithelial cells, mammary myoepithelial cells and/or mammary progenitor cells); (b) cultivating the mixed population of mammary cells and plasma cells of (a) on the scaffold, to produce a monolayer of polarized mammary cells located adjacent to and above the plasma cells, wherein the plasma cells are located adjacent to and above the upper surface of the scaffold, wherein the upper surface is located adjacent to and above the lower surface of the scaffold, and wherein the polarized mammary cells comprise an apical surface and a basal surface, thereby producing a cell construct for producing the cultured milk product. In some embodiments, the mammary cells are primary mammary cells. In some embodiments, the mammary cells are derived from a cell culture. In some embodiments, the mammary epithelial cells, myoepithelial cells and/or mammary progenitor cells are isolated from bone marrow, spleen tissue, lymph node tissue, mammary explants from mammary tissue (e.g., breast, udder, teat tissue), or raw breastmilk. In some embodiments, the mammary cells comprise mammary epithelial cells. In some embodiments, the mammary cells, comprise mammary myoepithelial cells. In some embodiments, the mammary cells, comprise mammary progenitor cells. In some embodiments, the plasma cells are isolated from any suitable human tissue or a cell culture. In some embodiments, the mammary cells and plasma cells are deposited concurrently. In some embodiments, the plasma cells are deposited onto the surface of the scaffold prior to the deposition of the mammary cells.

[0122] In some embodiments, the method comprises (a) depositing (i) isolated immortalized mammary epithelial cells, mammary myoepithelial cells and/or mammary progenitor cells, and (ii) isolated plasma cells on the upper surface of a scaffold having an upper surface and lower surface to produce a mixed population of plasma cells and immortalized mammary cells (i.e., immortalized mammary epithelial cells, immortalized mammary myoepithelial cells and/or immortalized mammary progenitor cells); (b) cultivating the mixed population of immortalized mammary cells and plasma cells of (a) on the scaffold, to produce a monolayer of polarized immortalized mammary cells located adjacent to and above the plasma cells, wherein the plasma cells are located adjacent to and above the upper surface of the scaffold, wherein the upper surface is located adjacent to and above the lower surface of the scaffold, and wherein the polarized immortalized mammary cells comprise an apical surface and a basal surface, thereby producing a cell construct for producing the cultured milk product. In some embodiments, the immortalized mammary cells comprise immortalized mammary epithelial cells. In some embodiments, the immortalized mammary cells, comprise immortalized mammary myoepithelial cells. In some embodiments, the immortalized mammary cells, comprise immortalized mammary progenitor cells. In some embodiments, the plasma cells are isolated from any suitable human tissue or a cell culture. In some embodiments, the immortalized mammary cells and plasma cells are deposited concurrently. In some embodiments, the plasma cells are deposited onto the surface of the scaffold prior to the deposition of the immortalized mammary cells. In certain embodiments, plasma cells are added to the culture of immortalized mammary epithelial cells to produce a co-culture of mammary cells and plasma cells. In certain embodiments, the plasma cells are cultivated with the immortalized mammary epithelial cells on the scaffold, thereby producing a cell construct for producing a cultured milk product with secretory products of the immune cells and mammary cells (e.g., sIgA). In certain embodiments, the isolated mammary cells are immortalized prior to co-culture of the cells.

[0123] In certain embodiments, the immune cells are stimulated to produce immunoglobins during co-culture. In certain embodiments, the immune cells produce one or more immunoglobins of a class selected from IgG, IgM and IgA. In certain embodiments the immune cells produce secretory IgA. Classes of immunoglobins produced by the immune cells include one or more IgA, IgM, and IgG. In certain embodiments, immune cells are co-cultured with MECs in a bioreactor according to methods described herein. In certain embodiments, the bioreactor is a hollow fiber bioreactor described herein.

[0124] In certain embodiments, mammary cells are modified and/or stimulated with prolactin according to the methods described herein to stimulate and optimize milk production. In certain embodiments, the mammary cells are modified to express a constitutively active prolactin receptor protein.

[0125] In certain embodiments, mammary cells are identified and isolated from mammary tissue samples. In some embodiments, the mammary cells are isolated and sorted via fluorescence-activated cell sorting, magnetic-activated cell sorting, and/or microfluidic cell sorting. In certain embodiments, the mammary epithelial cell populations are sorted by FACS analysis using markers known in the art for identifying the cell populations. In certain embodiments, myoepithelial mammary cells and luminal epithelial mammary cells are isolated by FACS analysis. In certain embodiments, progenitor myoepithelial mammary cells and/or progenitor luminal epithelial mammary cells are isolated by FACS analysis. Any suitable method known in the art for sorting mammary epithelial cells (e.g., luminal epithelial cells), myoepithelial cells, progenitor cells, and immune cells can be used. For example, mammary cells can be sorted using CD24, EPCAM and/or CD49f, cell surface markers.

[0126] In some embodiments, plasma cells are identified and isolated from primary mucosal tissue (e.g., oronasal, gastrointestinal, respiratory or mammary). In some embodiments, plasma cells are identified and isolated from primary mammary tissue samples. In some embodiments, the plasma cells are isolated and sorted via fluorescence-activated cell sorting, magnetic-activated cell sorting, and/or microfluidic cell sorting. In certain embodiments, plasma cells are sorted and isolated by FACS analysis. In certain embodiments plasma cells, plasmablasts, or pre-plasmablasts are sorted and isolated by FACS analysis using markers known in the art (e.g., CD20, CD38, CD138 and/or CD19).

[0127] In some embodiments, the culturing and/or cultivating of the mammary cells and plasma cells for the cell construct is carried out at a temperature of about 35°C to about 39°C (e.g., a temperature of about 35°C, 35.5°C, 36°C, 36.5°C, 37°C, 37.5°C, 38°C, 38.5°C or about 39°C, or any value or range therein, e.g., about 35°C to about 38°C, about 36°C to about 39°C, about 36.5°C to about 39°C, about 36.5°C to about 37.5°C, or about 36.5°C to about 38°C). In some embodiments, the culturing and/or cultivating is carried out at a temperature of about 37°C.

[0128] In some embodiments, the culturing and/or cultivating of the mammary cells and plasma cells for the cell construct is carried out at an atmospheric concentration of CO₂ of about 4% to about 6%, e.g., an atmospheric concentration of CO₂ of about 4%, 4.25%, 4.5%, 4.75%, 5%, 5.25%, 5.5%, 5.75%, or 6% or any value or range therein, e.g., about 4% to about 5.5%, about 4.5% to about 6%, about 4.5% to about 5.5%, or about 5% to about 6%). In some embodiments, the culturing and/or cultivating is carried out at an atmospheric concentration of CO₂ of about 5%.

[0129] In some embodiments, the culturing and/or cultivating of the mammary cells and the plasma cells for the cell construct comprises culturing and/or cultivating in a culture medium that is exchanged about every day to about every 10 days (e.g., every 1 day, every 2 days, every 3 days, every 4 days, every 5 days, every 6 days, every 7 days, every 8 days, every 9 days, every 10 days, or any value or range therein, e.g., about every day to every 3 days, about every 3 days to every 10

days, about every 2 days to every 5 days). In some embodiments, the culturing and/or cultivating further comprises culturing in a culture medium that is exchanged about every day to about every few hours to about every 10 days, e.g., about every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours to about every 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 days or any value or range therein. For example, in some embodiments, the culturing and/or cultivating further comprises culturing and/or cultivating in a culture medium that is exchanged about every 12 hours to about every 10 days, about every 10 hours to about every 5 days, or about every 5 hours to about every 3 days.

[0130] In some embodiments, the cell construct is stored in a freezer or in liquid nitrogen. The storage temperature depends on the desired storage length. For example, freezer temperature (e.g., storage at a temperature of about 0°C to about -80°C or less, e.g., about 0°C, -10°C, -20°C, -30°C, -40°C, -50°C, -60°C, -70°C, -80°C, -90°C, -100°C or any value or range therein) may be used if the cells are to be used within 6 months (e.g., within 1, 2, 3, 4, 5, or 6 months). For example, liquid nitrogen may be used (e.g., storage at a temperature of -100°C or less (e.g., about -100°C, -110°C, -120°C, -130, -140, -150, -160, -170, -180, -190°C, -200°C, or less) for longer term storage (e.g., storage of 6 months or longer, e.g., 6, 7, 8, 9, 10, 11, or 12 months, or 1, 2, 3, 4, 5, 6 or more years).

Cultured Milk Products

[0131] The present disclosure relates to cultured milk products that comprise protein, lipid, and oligosaccharide components and component concentrations that mimic human breast milk as produced by a lactating female, which compositions are produced, at least in part, by *in vitro* and/or *ex vivo* cultured mammary cells.

[0132] In some embodiments, two compositionally defined products can be obtained depending on the cellular inputs and culture conditions: (a) a functional nutrition product representing the biosynthetic output of cultured mammary epithelial cells (immortalized or from primary tissue samples); and (b) a similar product further comprising immunoglobulins, produced when plasma cells (PCs) are co-cultured with mammary epithelial cells. Plasma cells can be derived from a primary mammary tissue sample or, e.g., from a plasma cell line.

[0133] Contemplated product compositions of the present disclosure can be defined by total levels of protein, lipid, and carbohydrate (Tables 1A-1C) and/or by a signature of specific macronutrient components (Tables 2A-2C) present in concentrations and proportions consistent with human milk.

Table 1A. Macromolecular Composition of Functional Nutrition Products Collected from Human Mammary Epithelial Cells Cultured With or Without Plasma Cells.

Macromolecular Fraction	Concentration, g/L	%wt	Source
Protein	3-21 ^a	1-44	MECs
Total Immunoglobulin ^b	0.4-1.8	0.14-3.75	PCs
Lipid	9-135	3.1-95+	MECs
HMO	3.5-21	1.2-44	MECs
Lactose	32-115	11-95+	MECs
Total macromolecular content	48-293		MECs ± PCs
Energy (kcal/L)	375-1725		

HMO, human milk oligosaccharide. MEC, mammary epithelial cell; PC, plasma cell.

^a Non-immunoglobulin protein content.

^b Formulations lacking immunoglobulin content can be derived from MEC culture in the absence of plasma cells. Formulations with immunoglobulin content can be obtained by coculture of MECs with plasmacytes.

^c Long-chain fatty acids linoleic acid and alpha-linoleic acid are not synthesized by mammalian cells and are supplemented in cell culture media.

Table 1B. Macromolecular Composition of Functional Nutrition Products Collected from Human Mammary Epithelial Cells Cultured With or Without Plasma Cells.

Macromolecular Fraction	Concentration, g/L	%wt	Source
Protein	4.5-17.5 ^a	1.8-24	MECs
Total Immunoglobulin ^b	0.6-1.5	0.25-2.1	PCs
Lipid	13.5-111	5.5-95+	MECs
HMO	5.25-17.5	2.2-24	MECs
Lactose	48-96	20-95+	MECs
Total macromolecular content	71.9-244		MECs ± PCs
Energy (kcal/L)	375-1438		

HMO, human milk oligosaccharide. MEC, mammary epithelial cell; PC, plasma cell.

^a Non-immunoglobulin protein content.

^b Formulations lacking immunoglobulin content can be derived from MEC culture in the absence of plasma cells. Formulations with immunoglobulin content can be obtained by co-culture of MECs with plasmacytes.

^c Long-chain fatty acids linoleic acid and alpha-linoleic acid are not synthesized by mammalian cells and are supplemented in cell culture media.

Table 1C. Macromolecular Composition of Functional Nutrition Products Collected from Human Mammary Epithelial Cells Cultured With or Without Plasma Cells.

Macromolecular Fraction	Concentration, g/L	%wt	%vol	Source
Protein	6-14 ^a	3-15	1	MECs
Total Immunoglobulin ^b	0.8-1.2	0.4-1.3	0.1	PCs
Lipid	18-89	9-92	4	MECs
HMO	7-14	4-15	2.4	MECs
Lactose	64-77	33-80	4.6	MECs
Total macromolecular content	95.8-195.2		10-15	MECs ± PCs
Energy (kcal/L)	500-1150			

HMO, human milk oligosaccharide. MEC, mammary epithelial cell; PC, plasma cell.

^aNon-immunoglobulin protein content.

^bFormulations lacking immunoglobulin content can be derived from MEC culture in the absence of plasma cells. Formulations with immunoglobulin content can be obtained by co-culture of MECs with plasmacytes.

^cLong-chain fatty acids linoleic acid and alpha-linoleic acid are not synthesized by mammalian cells and are supplemented in cell culture media.

[0134] In some embodiments, the concentrations of components indicated in Tables 1A-1C can vary, each individually, for example, by having a concentration that is greater than that indicated by 0.1 fold, or 0.2 fold, or 0.3 fold, or 0.4 fold, or 0.5 fold, or 0.6 fold, or 0.7 fold, or 0.8 fold, or 0.9 fold, or 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 fold.

[0135] In some embodiments, the concentrations of components indicated in Tables 1A-1C can vary, each individually, for example, by having a concentration that is less than that indicated by 0.1 fold, or 0.2 fold, or 0.3 fold, or 0.4 fold, or 0.5 fold, or 0.6 fold, or 0.7 fold, or 0.8 fold, or 0.9 fold.

[0136] In some embodiments, milk products are contemplated herein that include a subset of the components (i.e., macromolecular fractions) of Tables 1A-1C. In other embodiments, milk products contemplated herein can exclude one or more of the components (i.e., macromolecular fractions) of Tables 1A-1C.

Table 2A. Macromolecular Content and Concentration Ranges of Functional Nutrition Products Derived from Human Mammary Epithelial Cells Cultured With or Without Plasma Cells.

Macromolecular Fraction	Concentration, g/L
Protein	
P-Casein	0.25- 1.9
K-Casein	0.25-0.9
a-Casein	0.05-0.75

a-Lactalbumin	1.35-4.9
Lysozyme	0.2-0.75
Lactoferrin	0.5-3
Haptocorrin	0.03-1.1
Butyrophilin	0.02-0.075
Osteopontin	0.025-0.3
Mucin MC5	0.25-0.9
Mucin BrE3	0.25-1.1
Lactadherin	0.03-1.1
Immunoglobulins	0.1-3
Secretory IgA	0.1-1.5
	0.07-2.4
	0.02-0.45
Total IgA Total IgG Total IgM	0.005-0.2
Lipids	
Saturated fatty acids	2.5-51
Palmitic acid (C16:0)	2-27
Stearic acid (C18:0)	0.35-7.5
Lauric acid (C12:0)	0.25-7.5
Monounsaturated fatty acids	3.5-69
Oleic acid (C18:1 n-9 Z)	3.5-68
Polyunsaturated fatty acids	1-30
Linoleic acid, LA	1-29
(C18:2 n-6 Z)	0.25-1.1
a-Linolenic acid, ALA (C18:3 n-3)	0.25-1.1
Eicosadienoic acid (C20:2)	0.25-1.1
Arachidonic acid, AA (C20:4 n-6)	0.15-8
Dihomo-γ-linolenic acid, DGLA (C20:3 n-6)	0.01-0.6
Docosahexadienoic acid, DHA (C22:6 n-3)	0.045-0.22
Cholesterol	0.05-0.6
Phospholipids, plasmalogens, sphingolipids	
Human Milk Oligosaccharides	0.8-4.8
Neutral	0.8-4.8
TF-LNH (trifucosyllacto-N-hexose) 2'-FL (2'-fucosyllactose)	0.8-4.8
DF-LNHII (difucosyllacto-N-hexaose)	0.4-2.4
LNFP I (1acto-A-fucopentaose I)	0.16-2.4
LNDFHI (lacto-N-difucosylhexaose I)	0.23-1.8
LNT (lacto-A-tetraose)	0.4-1.8
LNnT (lacto-A-neotetraose)	0.08-1.2
DF-L (Difucosyllactose) 3-FL (3-fucosyllactose)	0.16-1.8
Acidic	0.16-1.44
6'-SL (6'-sialyllactose)	0.04-1.2
DS-LNT (disialyllacto-A-tetraose)	0.04-0.84
FS-LNnH I (fucosyl-sialyl-lacto-N-neohexaose I) LST c	0.04-0.84
(sialyl-lacto-A-tetraose c)	0.04-0.84
3'-SL (3'-sialyllactose)	0.08-0.36
Lactose	34-104

Table 2B. Macromolecular Content and Concentration Ranges of Functional Nutrition Products Derived from Human Mammary Epithelial Cells Cultured With or Without Plasma Cells.

Macromolecular Fraction	Concentration, g/L
Protein	
B-Casein	0.38-1.9
K-Casein	0.38-0.75
a-Casein	0.08-0.63
a-Lactalbumin	2.0-4.13
Lysozyme	0.15-0.63
Lactoferrin	0.8-2.5
Haptocorrin	0.05-0.89
Butyrophilin	0.02-0.063
Osteopontin	0.04-0.25
Mucin MC5	0.38-0.75
Mucin BrE3	0.38-0.89
Lactadherin	0.045-0.09
Immunoglobulins	0.15-2.5
Secretory IgA	0.15-1.25
Total IgA	0.11-2
Total IgG	0.02-0.38
Total IgM	0.0075-0.13
Lipids	
Saturated fatty acids	3.75-43
Palmitic acid (C16:0)	2.25-23
Stearic acid (C18:0)	0.53-6.3
Lauric acid (C12:0)	0.38-6.3
Monounsaturated fatty acids	5.25-58
Oleic acid (C18:1 n-9 Z)	5.3-56
Polyunsaturated fatty acids	1.5-25
Linoleic acid, LA (C18:2 n-6 Z)	1.5-25
a-Linolenic acid, ALA (C18:3 n-3)	0.38-0.89
Eicosadienoic acid (C20:2)	0.38-0.89
Arachidonic acid, AA (C20:4 n-6)	0.38-0.89
Dihomo-γ-linolenic acid, DGLA (C20:3 n-6)	0.23-0.63

Docosahexadienoic acid, DHA (C22:6 n-3)	0.015-0.5
Cholesterol	0.07-0.19
Phospholipids, plasmalogens, sphingolipids	0.075-0.5
Human Milk Oligosaccharides	
Neutral	
TF-LNH (trifucosyllacto-N-hexose)	0.9-4.4
2'-FL (2'-fucosyllactose)	0.9-4.4
DF-LNHII (difucosyllacto-N-hexaose)	0.9-4.4
LNFP I (1acto-A-fucopentaose I)	0.45-2.2
LNDFHI (lacto-N-difucosylhexaose I)	0.18-2.2
LNT (lacto-A-tetraose)	0.27-1.7
LNnT (lacto-A-neotetraose)	0.4-1.7
DF-L (Difucosyllactose)	0.09-1.1
3-FL (3-fucosyllactose)	0.18-1.7
Acidic	
6'-SL (6'-sialyllactose)	0.18-1.3
DS-LNT (disialyllacto-A-tetraose)	0.04-1.1
FS-LNnH I (fucosyl-sialyl-lacto-N-neohexaose I)	0.04-0.8
LST c (sialyl-lacto-A-tetraose c)	0.04-0.8
3'-SL (3'-sialyllactose)	0.09-0.33
Lactose	50-98

Table 2C. Macromolecular Content and Concentration Ranges of Functional Nutrition Products Derived from Human Mammary Epithelial Cells Cultured With or Without Plasma Cells.

Macromolecular Fraction	Concentration, g/L
Protein	
B-Casein	0.5-1.5
K-Casein	0.5-0.6
a-Casein	0.1-0.5
a-Lactalbumin	2.7-3.3
Lysozyme	0.2-0.5
Lactoferrin	1-2
Haptocorrin	0.07-0.7
Butyrophilin	0.03-0.05
Osteopontin	0.05-0.2

Mucin MC5	0.5-0.6
Mucin BrE3	0.5-0.7
Lactadherin	0.06-0.07
Immunoglobulins	0.2-2
Secretory IgA	0.2-1.0
Total IgA	0.15-1.6
Total IgG	0.03-0.3
Total IgM	0.01-0.1
Lipids	
Saturated fatty acids	5-34
Palmitic acid (C16:0)	3-18
Stearic acid (C18:0)	0.7-5
Lauric acid (C12:0)	0.5-5
Monounsaturated fatty acids	7-46
Oleic acid (C18:1 n-9 Z)	7-45
Polyunsaturated fatty acids	2-20
Linoleic acid, LA (C18:2n-6 Z)	2-19
α -Linolenic acid, ALA (C18:3 n-3)	0.5-0.7
Eicosadienoic acid (C20:2)	0.5-0.7
Arachidonic acid, AA (C20:4 n-6)	0.5-0.7
Dihomo- γ -linolenic acid, DGLA (C20:3 n-6)	0.3-0.5
Docosahexadienoic acid, DHA (C22:6 n-3)	0.02-0.4
Cholesterol	0.09-0.15
Phospholipids, plasmalogens, sphingolipids	0.1-0.4
Human Milk Oligosaccharides Neutral	
TF-LNH (trifucosyllacto-N-hexose)	1-4
2'-FL (2'-fucosyllactose)	1-4
DF-LNHII (difucosyllacto-N-hexaose)	1-4
LNFP I (1acto-A-fucopentaose I)	0.5-2
LNDFHI (lacto-N-difucosylhexaose I)	0.2-2
LNT (lacto-A-tetraose)	0.3-1.5
LNnT (lacto-A-neotetraose)	0.5-1.5
DF-L (Difucosyllactose)	0.1-1
3-FL (3-fucosyllactose) Acidic	0.2-1.5
6'-SL (6'-sialyllactose)	0.2-1.2
DS-LNT (disialyllacto-A-tetraose)	0.05-1

FS-LNnH I (fucosyl-sialyl-lacto-N-neohexaose I)	0.05-0.7
LST c (sialyl-lacto-A-tetraose c)	0.05-0.7
3'-SL (3'-sialyllactose)	0.1-0.3
Lactose	67-78

[0137] In some embodiments, the concentrations of components indicated in Tables 2A-C can vary, each individually, for example, by having a concentration that is greater than that indicated by 0.1 fold, or 0.2 fold, or 0.3 fold, or 0.4 fold, or 0.5 fold, or 0.6 fold, or 0.7 fold, or 0.8 fold, or 0.9 fold, or 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 fold.

[0138] In some embodiments, the concentrations of components indicated in Tables 2A-C can vary, each individually, for example, by having a concentration that is less than that indicated by 0.1 fold, or 0.2 fold, or 0.3 fold, or 0.4 fold, or 0.5 fold, or 0.6 fold, or 0.7 fold, or 0.8 fold, or 0.9 fold.

[0139] In some embodiments, milk products are contemplated herein that include a subset of the components (i.e., macromolecular fractions) of Tables 2A-C. In other embodiments, milk products contemplated herein can exclude one or more of the components (i.e., macromolecular fractions) of Tables 2A-C.

[0140] In some embodiments, milk products are contemplated herein that further comprise serum albumin. In some embodiments, the serum albumin can have a concentration of about 0.025-3.5 g/L. In some embodiments, the serum albumin can have a concentration of about 0.01-2 g/L. In some embodiments, the serum albumin can have a concentration of about 0.15-1 g/L, and in some embodiments, the serum albumin can have a concentration of about 0.2-0.7 g/L.

[0141] One aspect of the present disclosure is directed to a milk product comprising about 6-14 grams per liter (g/L) protein components, about 18-89 g/L lipid components, about 7-14 g/L human milk oligosaccharides (HMOs), and about 64-77 g/L lactose, wherein at least one of the protein components, lipid components, HMOs, and lactose is produced by cultured human mammary epithelial cells.

[0142] In some embodiments, the protein component comprises about 55-65% dry weight of the milk product. In some embodiments, the protein component comprises one or more of beta-casein, kappa-casein and alpha-casein, and in some embodiments, the beta-casein can have a concentration of about 0.5-1.5 g/L, the kappa-casein can have a concentration of about 0.5-0.6 g/L and the alpha-casein can have a concentration of about 0.1-0.5 g/L in the milk product. In some embodiments of the milk product, the beta-casein, kappa-casein and alpha-casein together comprise about 35-45% dry weight percent of the protein component. In some embodiments, the beta-casein comprises greater than about 50% of total casein content.

[0143] In some embodiments, the protein component further comprises, for example, one or more of alpha-lactalbumin, lysozyme, lactoferrin, haptocorrin, butyrophilin, osteopontin, mucin MC5, mucin BrE3, and lactadherin. Some embodiments further comprise serum albumin. In some embodiments of the milk product, the alpha-lactalbumin can have a concentration of about 2.7-3.3 g/L, and in some embodiments, the lysozyme can have a concentration of about 0.2-0.5 g/L, and in some embodiments, the lactoferrin can have a concentration of about 1.0-2.0 g/L. In some embodiments, the haptocorrin can have a concentration of about 0.07-0.7 g/L, and in some embodiments, the butyrophilin can have a concentration of about 0.03-0.05 g/L. In some embodiments, the osteopontin can have a concentration of about 0.05-0.2 g/L, and in some embodiments, the mucin MC5 can have a concentration of about 0.5-0.6 g/L. In some embodiments, the mucin BrE3 can have a concentration of about 0.5-0.7 g/L, and in some embodiments, the lactadherin can have a concentration of about 0.06-0.07 g/L. In some embodiments serum albumin can have a concentration of about 0.025-3.5 g/L, or about 0.01-2 g/L, or about 0.15-1 g/L, or about 0.2-0.7 g/L.

[0144] In some embodiments of the milk product, the protein components are of human origin.

[0145] In some embodiments, the lipid component comprises one or more of saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, cholesterol, phospholipids, plasmalogens, and sphingolipids. In some embodiments of the milk product, the saturated fatty acids can have a concentration of about 5-34 g/L. The saturated fatty acids can comprise, for example, one or more of palmitic acid, stearic acid and lauric acid and combinations thereof. In some embodiments, the palmitic acid comprises at least about 50% sn-2 form. In some embodiments of the milk product, palmitic acid can have a concentration of about 3-18 g/L, in some embodiments, stearic acid can have a concentration of about 0.7-5 g/L, and in some embodiments, lauric acid can have a concentration of about 0.5-5 g/L.

[0146] In some embodiments, monounsaturated fatty acids can have a concentration of about 7-46 g/L of the milk product. The saturated fatty acid component can comprise, for example, oleic acid, in some embodiments, the oleic acid comprises at least about 50% sn-1 form, and in some embodiments, oleic acid can have a concentration of about 7-45 g/L in the milk product.

[0147] In some embodiments of the milk product, polyunsaturated fats can have a concentration of about 2-20 g/L. The polyunsaturated fats can comprise, for example, one or more of linoleic acid, alpha-linolenic acid, eicosadienoic acid, arachidonic acid, dihomo-gamma-linolenic acid, and docosahexadienoic acid. In some embodiments, the linoleic acid comprises at least about 50% sn-3 form, and in some embodiments, linoleic acid can have a concentration of about 2-19 g/L, and in some embodiments, alpha-linolenic acid can have a concentration of about 0.5-0.7 g/L. In some embodiments, eicosadienoic acid can have a concentration of about 0.5-0.7 g/L, and in some embodiments arachidonic acid can have a concentration of about 0.5-0.7 g/L. In some embodiments, dihomo-gamma-linolenic acid can have a concentration of about 0.3-0.5 g/L, and in some

embodiments, docosahexadienoic acid can have a concentration of about 0.02-0.4 g/L. Some embodiments of the milk product comprise all of the above polyunsaturated fats, wherein linoleic acid can have a concentration of about 2-19 g/L, alpha-linolenic acid can have a concentration of about 0.5-0.7 g/L, eicosadienoic acid can have a concentration of about 0.5-0.7 g/L, arachidonic acid can have a concentration of about 0.5-0.7 g/L, dihomogamma-linolenic acid can have a concentration of about 0.3-0.5 g/L, and docosahexadienoic acid can have a concentration of about 0.02-0.4 g/L in the milk product.

[0148] In some embodiments, cholesterol can have a concentration of about 0.09-0.15 g/L in milk product, and in some embodiments, phospholipids, plasmalogens, and sphingolipids together can have a concentration of about 0.1-0.4 g/L in the milk product.

[0149] In some embodiments, the milk product comprises one or more neutral oligosaccharides, one or more acidic oligosaccharides or one or more each of neutral oligosaccharides and acidic oligosaccharides.

[0150] In some embodiments, the one or more neutral oligosaccharides comprise TF-LNH (trifucosyllacto-N-hexose), 2'-FL (2'-fucosyllactose), DF-LNHII (difucosyllacto-N-hexaose), LNFP I (lacto-N-fucopentaose I), LNDFHI (lacto-N-difucosylhexaose I), LNT (lacto-N-tetraose), LNnT (lacto-N-neotetraose), DF-L (Difucosyllactose), and 3-FL (3-fucosyllactose).

[0151] In some embodiments, the one or more acidic oligosaccharides comprise 6'-SL (6'-sialyllactose), DS-LNT (disialyllacto-N-tetraose), FS-LNnH I (fucosyl-sialyl-lacto-N-neohexaose I), LST c (sialyl-lacto-N-tetraose c), and 3'-SL (3'-sialyllactose).

[0152] In some embodiments, the one or more neutral oligosaccharides comprises TF-LNH (trifucosyllacto-N-hexose), which oligosaccharide can have a concentration of about 1-4 g/L in the milk product. In some embodiments, the one or more neutral oligosaccharides comprises 2'-FL (2'-fucosyllactose), which oligosaccharide can have a concentration of about 1-4 g/L in the milk product, and in some embodiments, the one or more neutral oligosaccharides comprises DF-LNH II (difucosyllacto-N-hexaose), which oligosaccharide can have a concentration of about 1-4 g/L in the milk product. In some embodiments, the one or more neutral oligosaccharides comprises LNFP I (lacto-N-fucopentaose I), which oligosaccharide can have a concentration of about 0.5-2 g/L in the milk product, and in some embodiments, the one or more neutral oligosaccharides comprises LNDFH I (lacto-N-difucosylhexaose I), which oligosaccharide can have a concentration of about 0.2-2 g/L in the milk product. In some embodiments, the one or more neutral oligosaccharides comprises LNT (lacto-N-tetraose), which oligosaccharide can have a concentration of about 0.3-1.5 g/L in the milk product, and in some embodiments, the one or more neutral oligosaccharides comprises LNnT (lacto-N-neotetraose), which oligosaccharide can have a concentration of about 0.5-1.5 g/L in the milk product. In some embodiments, the one or more neutral oligosaccharides comprises DF-L (Difucosyllactose), which oligosaccharide can have a concentration of about 0.1-1

g/L in the milk product, and in some embodiments, the one or more neutral oligosaccharides comprises 3-FL (3-fucosyllactose), which oligosaccharide can have a concentration of about 0.2-1.5 g/L of the milk product.

[0153] In some embodiments, the one or more acidic oligosaccharides comprises 6'-SL (6'-sialyllactose), which oligosaccharide can have a concentration of about 0.2-1.2 g/L in the milk product, and in some embodiments, the one or more acidic oligosaccharides comprises DS-LNT (disialyllacto-N-tetraose), which oligosaccharide can have a concentration of about 0.05-1 g/L in the milk product. In some embodiments, the one or more acidic oligosaccharides comprises FS-LNnH I (fucosyl-sialyl-lacto-N-neohexaose I), which oligosaccharide can have a concentration of about 0.05-0.7 g/L in the milk product, and in some embodiments, the one or more acidic oligosaccharides comprises LST c (sialyl-lacto-N-tetraose c), which oligosaccharide can have a concentration of about 0.05-0.7 g/L in the milk product. In some embodiments, the one or more acidic oligosaccharides comprises 3'-SL (3'-sialyllactose), which oligosaccharide can have a concentration of about 0.1-0.3 g/L in the milk product.

[0154] In some embodiments of the milk product the one or more neutral oligosaccharides comprise TF-LNH (trifucosyllacto-N-hexose), 2'-FL (2'-fucosyllactose), DF-LNH II (difucosyllacto-N-hexaose), LNFP I (lacto-N-fucopentaose I), LNDFHI (lacto-N-difucosylhexaose I), LNT (lacto-N-tetraose), LNnT (lacto-N-neotetraose), DF-L (Difucosyllactose), and 3-FL (3-fucosyllactose) and the one or more acidic oligosaccharides comprises 6'-SL (6'-sialyllactose), DS-LNT (disialyllacto-N-tetraose), FS-LNnH I (fucosyl-sialyl-lacto-N-neohexaose I), LST c (sialyl-lacto-N-tetraose c), and 3'-SL (3'-sialyllactose).

[0155] In some embodiments, the milk product comprises about 1-4 g/L TF-LNH (trifucosyllacto-N-hexose), comprises about 1-4 g/L 2'-FL (2'-fucosyllactose), comprises about 1-4 g/L DF-LNH II (difucosyllacto-N-hexaose), comprises about 0.5-2 g/L LNFP I (lacto-N-fucopentaose I), comprises about 0.2-2 g/L LNDFH I (lacto-N-difucosylhexaose I), comprises about 0.3-1.5 g/L LNT (lacto-N-tetraose), comprises about 0.5-1.5 g/L LNnT (lacto-N-neotetraose), comprises about 0.1-1 g/L DF-L (Difucosyllactose), comprises about 0.2-1.5 g/L 3-FL (3-fucosyllactose), comprises about 0.2-1.2 g/L 6'-SL (6'-sialyllactose), comprises about 0.05-1 g/L DS-LNT (disialyllacto-N-tetraose), comprises about 0.05-0.7 g/L FS-LNnH I (fucosyl-sialyl-lacto-N-neohexaose I), comprises about 0.05-0.7 g/L LST c (sialyl-lacto-N-tetraose c), and comprises about 0.1-0.3 g/L 3'-SL (3'-sialyllactose).

[0156] In some embodiments, the neutral oligosaccharides content comprises at least about 2-fold, or about 3-fold, or about 4-fold, or about 5-fold, or about 6-fold, or about 7-fold, or about 8-fold, or about 9-fold, or about 10-fold, or about 11-fold, or about 12-fold, or about 13-fold, or about 14-fold, or about 15-fold more by weight than acidic oligosaccharide content.

[0157] In another aspect of the disclosure, a milk product is provided, comprising about 614 grams per liter (g/L) protein components, about 18-89 g/L lipid components, about 7-14 g/L human milk oligosaccharides (HMOs), and about 64-77 g/L lactose, wherein the protein components comprise one or more of beta-casein, kappa-casein, and alpha-casein, algalactalbumin, lysozyme, lactoferrin, haptocorrin, butyrophilin, osteopontin, mucin MC5, mucin BrE3, and lactadherin, wherein the lipid components comprise one or more of palmitic acid, stearic acid and lauric acid, oleic acid, linoleic acid, alpha-linolenic acid, eicosadienoic acid, arachidonic acid, dihomogamma-linolenic acid, docosahexadienoic acid, cholesterol, phospholipids, plasmalogens and sphingolipids, wherein the human milk oligosaccharides comprise one or more of TF-LNH (trifucosyllacto-N-hexose), 2'-FL (2'-fucosyllactose), DF-LNHII (difucosyllacto-N-hexaose), LNFP I (lacto-N-fucopentaose I), LNDFHI (lacto-N-difucosylhexaose I), LNT (lacto-N-tetraose), LNnT (lacto-N-neotetraose), DF-L (Difucosyllactose), and 3-FL (3-fucosyllactose), 6'-SL (6'-sialyllactose), DS-LNT (disialyllacto-N-tetraose), FS-LNnH I (fucosyl-sialyl-lacto-N-neohexaose I), LST c (sialyl-lacto-N-tetraose c), and 3'-SL (3'-sialyllactose), and wherein at least one of the protein components, lipid components, HMOs, and lactose is produced by cultured human mammary epithelial cells. In some embodiments, the milk product further comprises serum albumin.

[0158] In some embodiments, the milk product comprises about 0.5-1.5 g/L beta-casein, comprises about 0.5-0.6 g/L kappa-casein, comprises about 0.1-0.5 g/L alpha-casein, comprises about 2.7-3.3 g/L alpha-lactalbumin, comprises about 0.2-0.5 g/L lysozyme, comprises about 1.0-2.0 g/L lactoferrin, comprises about 0.07-0.7 g/L haptocorrin, comprises about 0.03-0.05 g/L butyrophilin, comprises about 0.05-0.2 g/L osteopontin, comprises about 0.5-0.6 g/L mucin MC5, comprises about 0.5-0.7 g/L mucin BrE3, comprises about 0.06-0.07 g/L lactadherin, comprises about 2-19 g/L linoleic acid, comprises about 0.5-0.7 g/L alpha-linolenic acid, comprises about 0.5-0.7 g/L eicosadienoic acid, comprises about 0.5-0.7 g/L arachidonic acid, comprises about 0.3-0.5 g/L dihomogamma-linolenic acid, comprises about 0.02-0.4 g/L docosahexadienoic acid, comprises about 0.09-0.15 g/L cholesterol, together comprise about 0.1-0.4 g/L phospholipids, plasmalogens and sphingolipids, comprises about 1-4 g/L TF-LNH (trifucosyllacto-N-hexose), comprises about 1-4 g/L 2'-FL (2'-fucosyllactose), comprises about 1-4 g/L DF-LNH II (difucosyllacto-N-hexaose), comprises about 0.5-2 g/L LNFP I (lacto-N-fucopentaose I), comprises about 0.2-2 g/L LNDFHI (lacto-N-difucosylhexaose I), comprises about 0.3-1.5 g/L LNT (lacto-N-tetraose), comprises about 0.5-1.5 g/L LNnT (lacto-N-neotetraose), comprises about 0.1-1 g/L DF-L (Difucosyllactose), comprises about 0.2-1.5 g/L 3-FL (3-fucosyllactose), comprises about 0.2-1.2 g/L 6'-SL (6'-sialyllactose), comprises about 0.05-1 g/L DS-LNT (disialyllacto-N-tetraose), comprises about 0.05-0.7 g/L FS-LNnH I (fucosyl-sialyl-lacto-N-neohexaose I), comprises about 0.05-0.7 g/L LST c (sialyl-lacto-N-tetraose c), and comprises about 0.1-0.3 g/L 3'-SL (3'-sialyllactose). In some embodiments, the milk product further comprises serum albumin, which serum albumin has a concentration of about 0.025-3.5 g/L, or about 0.01-2 g/L, or about 0.15-1 g/L, or about 0.3-0.7 g/L.

[0159] In another aspect of the disclosure, a milk product is provided comprising about 3-15 percent protein by weight, about 9-92 percent lipid by weight, about 4-15 percent by weight human milk oligosaccharides (HMOs), and about 33-80 percent by weight lactose, wherein at least one of the protein, lipid, HMOs, and lactose is produced by cultured human mammary epithelial cells.

[0160] In some embodiments of some aspects of the disclosure, the milk product comprises at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% of the overall macromolecular composition of human breast milk.

[0161] In some embodiments of the milk product of the disclosure, non-protein nitrogen content is at least about 10%, or at least about 15%, or at least about 20%, or at least about 25%, or at least about 30% of total nitrogen content.

[0162] In some embodiments, the milk product comprises about 500-1150 kcal/L available energy content, and in some embodiments, between about 40-55% of the available energy content is from lipids. In some embodiments, the milk product comprises between about 95.8 and 195.2 g/L macromolecular content.

[0163] In some embodiments, the cultured milk product comprises 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.

[0164] In some embodiments, the cultured milk product comprises Beta-casein, Alpha-lactalbumin, Kappa-casein, Alpha-S1-casein, Lactoferrin, Lactadherin, Lysozyme, C12:0 (lauric acid), C14:0 (myristic acid), C16:0 (palmitic acid), C18:2n-6 (linoleic acid, LA), C18:3n-3 (alpha-linolenic acid, ALA), Lactose, Glucose, 3'-SL, and 6'-SL.

[0165] In some embodiments, the cultured milk product comprises 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 (erucic acid), C24:1 (nervonic acid), Lactose, Glucose, 3'-SL, 6'-SL, LNT, and LNnT.

[0166] In some embodiments, the cultured milk products disclosed herein further comprise at least one oxylipin metabolite of a polyunsaturated fatty acid, or at least one endocannabinoid. In some embodiments, the oxylipin metabolite is 9-hydroxyoctadecadienoic acid (9-HODE); 13-hydroxyoctadecadienoic acid (13-HODE); 5,15-dihydroxyeicosatetraenoic acid (5,15-DiHETE);

17,18-dihydroxyicosatetraenoic 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)-epoxyicosatrienoic acid (14(15)-EpETrE); 19(20)-epoxydocosapentanoic acid (19(20)-EpDPE); 17-hydroxydocosahexaenoic acid (17-HDoHE); 5-hydroxyicosatetraenoic acid (5-HETE); 8-hydroxyicosatetraenoic acid (8-HETE); 9-hydroxyicosatetraenoic acid (9-HETE); 11-hydroxyicosatetraenoic acid (11-HETE); 12-hydroxyicosatetraenoic 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-epoxyicosatetraenoic acid (17(18)-EpETE); 6-Keto-prostaglandin F1 α (6-keto-PGF1 α); or 15(S)-Hydroxyicosatrienoic acid (15(S)-HETrE). In some embodiments, the endocannabinoid is anandamide or 2-arachidonoylglycerol (2-AG).

[0167] In some embodiments, the cultured milk product further comprises a preservative, stabilizer, antioxidant, emulsifier, or thickener. In some embodiments, the cultured milk product further comprises lecithin. In some embodiments, the cultured milk product further comprises carrageenan. In some embodiments, the cultured milk product further comprises beta carotene. In some embodiments, the cultured milk product further comprises vitamin E. In some embodiments, the cultured milk product further comprises ascorbyl palmitate or ascorbic acid. In some embodiments, the cultured milk product further comprises lecithin, ascorbyl palmitate and vitamin E. In some embodiments, the cultured milk product further comprises lecithin, ascorbic acid and vitamin E.

[0168] In another aspect of the disclosure, a containerized milk product is provided, comprising the milk product according to some embodiments of the disclosure that is packaged into a container.

[0169] In another aspect of the disclosure, a frozen milk product is provided, comprising the milk product according to some embodiments of the disclosure that has been frozen. In some embodiments, the frozen milk product is packaged into a container making a containerized frozen milk product. In some embodiments, the cultured milk product is stored at about -18°C to about -80°C. In some embodiments, the cultured milk product is stored at about -18°C. In some embodiments, the cultured milk product is stored at about -20°C. In some embodiments, the cultured milk product is stored at about -80°C.

[0170] In another aspect of the disclosure, a lyophilized milk product is provided, comprising the milk product according to some embodiments of the disclosure that has been lyophilized. In some embodiments, the lyophilized milk product is packaged into a container making a containerized lyophilized milk product.

[0171] In another aspect of the disclosure, an extracted milk product is provided, comprising one or more components extracted from the milk product according to some embodiments of the disclosure. Non-limiting examples of components that can be extracted from the milk product include protein, lipid, carbohydrate, vitamin, and/or mineral content. In some embodiments, the one or more

components extracted from the collected milk product are lyophilized or concentrated to produce a lyophilized or a concentrated extracted milk product component. In some embodiments, the one or more components extracted from the collected milk product are concentrated by membrane filtration or reverse osmosis, while in other embodiments, the whole, un-extracted milk product is concentrated by membrane filtration or reverse osmosis. In some embodiments, the one or more extracted components from the collected milk product comprise milk protein, lipid, carbohydrate, vitamin, and minerals. In some embodiments, the one or more extracted milk product components are packaged in a container, and in some embodiments, concentrated whole, un-extracted milk product is packaged in a container.

[0172] In some embodiments, the container is sterile, vacuum-sealed, or classified as food grade, or any combination thereof. Non-limiting examples of containers comprise a canister, a jar, a bottle, a bag, a box, or a pouch. In some embodiments, the container is a canister, a jar, a bottle, a bag, a box, or a pouch.

Immunoglobulins

[0173] In some embodiments, the cultured milk product further comprises one or more immunoglobulins or sIgA. In some embodiments, the cultured milk product comprises one or more of IgA, IgG, and IgM. In some embodiments, the cultured milk product comprises IgA2 (secretory) and IgA1 (non-secretory). In some embodiments, the plasma cells of the cell construct produce IgA and the mammary epithelial cells process the IgA to yield sIgA (IgA2). sIgA comprises a secretory component, the extracellular domain of the polymeric Ig receptor, attached to an IgA. Mammary epithelial cells process IgA by cleaving the extracellular domain of a polymeric Ig receptor to generate sIgA. In some embodiments, sIgA is secreted from the apical surface of the mammary epithelial cell.

[0174] In certain embodiments, the immunoglobulins bind to an antigen of a microorganism (i.e., bacterium or virus). In certain embodiments, the immunoglobulins bind to viral or bacterial antigens capable of causing an infectious disease in humans. In certain embodiments, the immunoglobulins bind to viral or bacterial antigens that cause infections of respiratory or gastrointestinal epithelium. In certain embodiments, the immunoglobulins bind antigens from microorganisms that cause enterocolitis or sepsis in infants.

[0175] In certain embodiments, the milk product comprises about 0.2-1.0 g/L secretory IgA. In certain embodiments, the milk product comprises about 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, or 1.0 g/L secretory IgA. In certain embodiments, the milk product comprises about 0.15-1.6 g/L total IgA. In certain embodiments, the milk product comprises about 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.7, 0.75, 0.8, 0.85, 0.9, 0.95, 1.0, 1.05, 1.1, 1.15, 1.2, 1.25, 1.3, 1.35, 1.4, 1.45, 1.5, 1.55, or 1.6 g/L total IgA.

[0176] In some embodiments that comprise IgG, the milk product comprises about 0.03-0.3 g/L IgG. In certain embodiments, that comprise IgG, the milk product comprises about 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.2, 0.21, 0.22, 0.23, 0.24, 0.25, 0.26, 0.27, 0.28, 0.29, 0.3 g/L. In some embodiments that comprise IgM, the milk product comprises about 0.01-0.1 g/L IgM. In certain embodiments, the milk product comprises about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.1 g/L IgM. In some embodiments, the milk product comprises about 0.2-2.0 percent by weight total immunoglobulins. In certain embodiments, the milk product comprises 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0 percent by weight total immunoglobulins.

[0177] Included herein are immunotherapeutic compositions comprising (a) IgA and sIgA derived from the secreted products of mammary epithelial cells co-cultured with plasma cells, and (b) a pharmaceutically acceptable excipient. In some embodiments, the IgA and sIgA are isolated from the product resulting from co-culturing mammary epithelial cells and plasma cells (e.g., a cultured milk product). In some embodiments, the pharmaceutically acceptable excipient is a stabilizer, a surfactant, a buffer or tonicity agent. In some embodiments, 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.

[0178] In some embodiments, IgA is produced by the plasma cells in the cell construct. The IgA binds to a receptor (polymeric Ig receptor) on the basal surface of mammary epithelial cells. The IgA and receptor are transported into the mammary epithelial cells. The mammary cells process the IgA. The extracellular domain of the receptor bound to the IgA (secretory component) is cleaved by a proteinase within the mammary epithelial cells and the IgA bound to secretory component is secreted from the apical surface of the mammary epithelial cells to yield sIgA. In some embodiments, the sIgA is secreted as part of the cultured milk product from the apical surface of the mammary cells into the apical compartment. In some embodiments, the sIgA is isolated from the cultured milk product. In some embodiments, the sIgA is not isolated from the cultured milk product.

Basal Culture Media and Lactogenic Media

[0179] In some embodiments, the culture medium comprises a carbon source, a chemical buffering system, one or more essential amino acids, one or more vitamins and/or cofactors, and one or more inorganic salts. In some embodiments, the carbon source, chemical buffering system, one or more essential amino acids, one or more vitamins and/or cofactors, and/or one or more inorganic salts are food grade.

[0180] In some embodiments, the culture medium is lactogenic culture medium. In some embodiments, the culture medium further comprises prolactin (e.g., mammalian prolactin, e.g.,

human prolactin), linoleic and alpha-linoleic acid, estrogen and/or progesterone. For example, in some embodiments, the culture medium comprises prolactin (or prolactin is added) in an amount from about 20 ng/mL to about 200 ng/mL of culture medium, e.g., about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 ng/mL or any value or range therein. In some embodiments, the culture medium comprises prolactin (or prolactin is added) in an amount from about 20 ng/mL to about 195 ng/mL, about 50 ng/mL to about 150 ng/mL, about 25 ng/mL to about 175 ng/mL, about 45 ng/mL to about 200 ng/mL, or about 75 ng/mL to about 190 ng/mL of culture medium. In some embodiments, the culture medium further comprises other factors to improve efficiency, including, but not limited to, insulin, an epidermal growth factor, and/or a hydrocortisone.

[0181] In some embodiments, the culture medium comprises a carbon source in an amount from about 1 g/L to about 15 g/L of culture medium (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 g/L or any value or range therein), or about 1, 2, 3, 4, 5 or 6 g/L to about 7, 8, 9, or 10, 11, 12, 13, 14 or 15 g/L of the culture medium. Non-limiting examples of a carbon source include glucose and/or pyruvate. For example, in some embodiments, the culture medium comprises glucose in an amount from about 1 g/L to about 12 g/L of culture medium, e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 g/L or any value or range therein. In some embodiments, the culture medium comprises glucose in an amount from about 1 g/L to about 6 g/L, about 4 g/L to about 12 g/L, about 2.5 g/L to about 10.5 g/L, about 1.5 g/L to about 11.5 g/L, or about 2 g/L to about 10 g/L of culture medium. In some embodiments, the culture medium comprises glucose in an amount from about 1, 2, 3, or 4 g/L to about 5, 6, 7, 8, 9, 10, 11, or 12 g/L or about 1, 2, 3, 4, 5, or 6 g/L to about 7, 8, 9, 10, 11, or 12 g/L. In some embodiments, the culture medium comprises pyruvate in an amount from about 5 g/L to about 15 g/L of culture medium, e.g., about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 g/L or any value or range therein. In some embodiments, the culture medium comprises pyruvate in an amount from about 5 g/L to about 14.5 g/L, about 10 g/L to about 15 g/L, about 7.5 g/L to about 10.5 g/L, about 5.5 g/L to about 14.5 g/L, or about 8 g/L to about 10 g/L of culture medium. In some embodiments, the culture medium comprises pyruvate in an amount from about 5, 6, 7, or 8 g/L to about 9, 10, 11, 12, 13, 14 or 15 g/L or about 5, 6, 7, 8, 9, or 10 g/L to about 11, 12, 13, 14 or 15 g/L.

[0182] In some embodiments, the culture medium comprises a chemical buffering system in an amount from about 1 g/L to about 4 g/L (e.g., about 1, 1.5, 2, 2.5, 3, 3.5, or 4 g/L or any value or range therein) of culture medium or about 10 mM to about 25 mM (e.g., about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 mM or any value or range therein). In some embodiments, the chemical buffering system includes, but is not limited to, sodium bicarbonate and/or 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). For example, in some embodiments, the culture medium comprises sodium bicarbonate in an amount from about 1 g/L to about 4 g/L of culture medium, e.g., about 1, 1.5, 2, 2.5, 3, 3.5, or 4 g/L or any value or range therein. In some embodiments, the culture medium comprises sodium bicarbonate in an amount from about 1 g/L to

about 3.75 g/L, about 1.25 g/L to about 4 g/L, about 2.5 g/L to about 3 g/L, about 1.5 g/L to about 4 g/L, or about 2 g/L to about 3.5 g/L of culture medium. In some embodiments, the culture medium comprises HEPES in an amount from about 10 mM to about 25 mM, e.g., about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 mM or any value or range therein. In some embodiments, the culture medium comprises HEPES in an amount from about 11 mM to about 25 mM, about 10 mM to about 20 mM, about 12.5 mM to about 22.5 mM, about 15 mM to about 20.75 mM, or about 10 mM to about 20 mM.

[0183] In some embodiments, the culture medium comprises one or more essential amino acids in an amount from about 0.5 mM to about 5 mM (e.g., about 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5 mM or any value or range therein) or about 0.5, 1, 1.5, 2 mM to about 2.5, 3, 3.5, 4, 4.5, or 5 mM. In some embodiments, the one or more essential amino acids is histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and/or arginine. For example, in some embodiments, the culture medium comprises arginine in an amount from about 0.5 mM to about 5 mM, e.g., about 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5 mM or any value or range therein. In some embodiments, the culture medium comprises an essential amino acids in an amount from about 0.5 mM to about 4.75 mM, about 2 mM to about 3.5 mM, about 0.5 mM to about 3.5 mM, about 1 mM to about 5 mM, or about 3.5 mM to about 5 mM.

[0184] In some embodiments, the culture medium comprises one or more vitamins and/or cofactors in an amount from about 0.01 μ M to about 50 μ M (e.g., about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 12.5, 15, 17.5, 20, 25, 30, 35, 40, 45, 46, 47, 48, 49, 49.025, 49.05, 49.075, or 50 μ M or any value or range therein) or about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, or 0.9 μ M to about 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 4, 5, 6 μ M or about 0.02, 0.025, 0.05, 0.075, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10 μ M to about 12.5, 15, 17.5, 20, 25, 30, 35, 40, 45, 46, 47, 48, 49, 49.025, 49.05, 49.075, or 50 μ M. In some embodiments, one or more vitamins and/or cofactors include, but are not limited to, thiamine and/or riboflavin. For example, in some embodiments, the culture medium comprises thiamine in an amount from about 0.025 μ M to about 50 μ M, e.g., about 0.025, 0.05, 0.075, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12.5, 15, 17.5, 20, 25, 30, 35, 40, 45, 46, 47, 48, 49, 49.025, 49.05, 49.075, or 50 μ M or any value or range therein. In some embodiments, the culture medium comprises thiamine in an amount from about 0.025 μ M to about 45.075 μ M, about 1 μ M to about 40 μ M, about 5 μ M to about 35.075 μ M, about 10 μ M to about 50 μ M, or about 0.05 μ M to about 45.5 μ M. In some embodiments, the culture medium comprises riboflavin in an amount from about 0.01 μ M to about 3 μ M, e.g., about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, or 3 μ M or any value or range therein. In some embodiments, the culture medium comprises

riboflavin in an amount from about 0.01 μM to about 2.05 μM , about 1 μM to about 2.95 μM , about 0.05 μM to about 3 μM , about 0.08 μM to about 1.55 μM , or about 0.05 μM to about 2.9 μM .

[0185] In some embodiments, the culture medium comprises one or more inorganic salts in an amount from about 100 mg/L to about 150 mg/L of culture medium (e.g., about 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, or 150 mg/L or any value or range therein) or about 100 mg/L to about 150 mg/L of culture medium (e.g., about 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, or 150 mg/L or any value or range therein). In some embodiments, one or more inorganic salts include, but are not limited to, calcium and/or magnesium. For example, in some embodiments, the culture medium comprises calcium in an amount from about 100 mg/L to about 150 mg/L of culture medium, e.g., about 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, or 150 mg/L or any value or range therein. In some embodiments, the culture medium comprises arginine in an amount from about 100 mg/L to about 125 mg/L, about 105 mg/L to about 150 mg/L, about 120 mg/L to about 130 mg/L, or about 100 mg/L to about 145 mg/L of culture medium. In some embodiments, the culture medium comprises magnesium in an amount from about 0.01 mM to about 1 mM, e.g., about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, or 1 mM or any value or range therein. In some embodiments, the culture medium comprises magnesium in an amount from about 0.05 mM to about 1 mM, about 0.01 mM to about 0.78 mM, about 0.5 mM to about 1 mM, about 0.03 mM to about 0.75 mM, or about 0.25 mM to about 0.95 mM.

[0186] In some embodiments, the culture medium comprises a carbon source in an amount from about 1 g/L to about 15 g/L of culture medium (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 g/L or any value or range therein), or about 1, 2, 3, 4, 5 or 6 g/L to about 7, 8, 9, or 10, 11, 12, 13, 14 or 15 g/L of the culture medium. In some embodiments, the carbon source includes, but is not limited to, glucose and/or pyruvate. For example, in some embodiments, the culture medium comprises glucose in an amount from about 1 g/L to about 12 g/L of culture medium, e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 g/L or any value or range therein. In some embodiments, the culture medium comprises glucose in an amount from about 1 g/L to about 6 g/L, about 4 g/L to about 12 g/L, about 2.5 g/L to about 10.5 g/L, about 1.5 g/L to about 11.5 g/L, or about 2 g/L to about 10 g/L of culture medium. In some embodiments, the culture medium comprises pyruvate at an amount of about 5 g/L to about 15 g/L of culture medium, e.g., about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 g/L or any value or range therein. In some embodiments, the culture medium comprises pyruvate in an amount from about 5 g/L to about 14.5 g/L, about 10 g/L to about 15 g/L, about 7.5 g/L to about 10.5 g/L, about 5.5 g/L to about 14.5 g/L, or about 8 g/L to about 10 g/L of culture medium.

[0187] In some embodiments, the culture medium comprises a chemical buffering system in an amount from about 1 g/L to about 4 g/L (e.g., about 1, 1.5, 2, 2.5, 3, 3.5, or 4 g/L or any value or

range therein) of culture medium or about 10 mM to about 25 mM (e.g., about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 mM or any value or range therein). In some embodiments, the chemical buffering system includes, but is not limited to, sodium bicarbonate and/or HEPES. For example, in some embodiments, the culture medium comprises sodium bicarbonate in an amount from about 1 g/L to about 4 g/L of culture medium, e.g., about 1, 1.5, 2, 2.5, 3, 3.5, or 4 g/L or any value or range therein. In some embodiments, the culture medium comprises sodium bicarbonate in an amount from about 1 g/L to about 3.75 g/L, about 1.25 g/L to about 4 g/L, about 2.5 g/L to about 3 g/L, about 1.5 g/L to about 4 g/L, or about 2 g/L to about 3.5 g/L of culture medium. In some embodiments, the culture medium comprises HEPES in an amount from about 10 mM to about 25 mM, e.g., about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 mM or any value or range therein. In some embodiments, the culture medium comprises HEPES in an amount from about 1 mM to about 25 mM, about 10 mM to about 20 mM, about 12.5 mM to about 22.5 mM, about 15 mM to about 20.75 mM, or about 10 mM to about 20 mM.

[0188] In some embodiments, the culture medium comprises one or more essential amino acids in an amount from about 0.5 mM to about 5 mM (e.g., about 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5 mM or any value or range therein) or about 0.5, 1, 1.5, 2 mM to about 2.5, 3, 3.5, 4, 4.5, or 5 mM. In some embodiments, one or more essential amino acids is arginine and/or cysteine. For example, in some embodiments, the culture medium comprises arginine in an amount from about 0.5 mM to about 5 mM, e.g., about 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5 mM or any value or range therein. In some embodiments, the culture medium comprises arginine in an amount from about 0.5 mM to about 4.75 mM, about 2 mM to about 3.5 mM, about 0.5 mM to about 3.5 mM, about 1 mM to about 5 mM, or about 3.5 mM to about 5 mM. For example, in some embodiments, the culture medium comprises cysteine in an amount from about 0.5 mM to about 5 mM, e.g., about 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5 mM or any value or range therein. In some embodiments, the culture medium comprises cysteine in an amount from about 0.5 mM to about 4.75 mM, about 2 mM to about 3.5 mM, about 0.5 mM to about 3.5 mM, about 1 mM to about 5 mM, or about 3.5 mM to about 5 mM.

[0189] In some embodiments, the culture medium comprises one or more vitamins and/or cofactors in an amount from about 0.01 μ M to about 50 μ M (e.g., about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 12.5, 15, 17.5, 20, 25, 30, 35, 40, 45, 46, 47, 48, 49, 49.025, 49.05, 49.075, or 50 μ M or any value or range therein) or about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, or 0.9 μ M to about 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 3, 4, 5, 6 μ M or about 0.02, 0.025, 0.05, 0.075, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10 μ M to about 12.5, 15, 17.5, 20, 25, 30, 35, 40, 45, 46, 47, 48, 49, 49.025, 49.05, 49.075, or 50 μ M. In some embodiments, one or more vitamins and/or cofactors includes, but is not limited to, thiamine and/or riboflavin. For example, in some embodiments, the culture medium comprises thiamine in an amount from about 0.025 μ M to about 50 μ M, e.g., 0.025, 0.05, 0.075, 1,

1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12.5, 15, 17.5, 20, 25, 30, 35, 40, 45, 46, 47, 48, 49, 49.025, 49.05, 49.075, or 50 μM or any value or range therein. In some embodiments, the culture medium comprises thiamine in an amount from about 0.025 μM to about 45.075 μM , about 1 μM to about 40 μM , about 5 μM to about 35.075 μM , about 10 μM to about 50 μM , or about 0.05 μM to about 45.5 μM . In some embodiments, the culture medium comprises riboflavin in an amount from about 0.01 μM to about 3 μM , e.g., 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, or 3 μM or any value or range therein. In some embodiments, the culture medium comprises riboflavin in an amount from about 0.01 μM to about 2.05 μM , about 1 μM to about 2.95 μM , about 0.05 μM to about 3 μM , about 0.08 μM to about 1.55 μM , or about 0.05 μM to about 2.9 μM .

[0190] In some embodiments, the culture medium comprises one or more inorganic salts in an amount from about 100 mg/L to about 150 mg/L of culture medium (e.g., about 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, or 150 mg/L or any value or range therein) or about 100 mg/L to about 150 mg/L of culture medium (e.g., about 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, or 150 mg/L or any value or range therein). In some embodiments, exemplary one or more inorganic salts is calcium and/or magnesium. For example, in some embodiments, the culture medium comprises calcium in an amount from about 100 mg/L to about 150 mg/L of culture medium, e.g., about 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, or 150 mg/L or any value or range therein. In some embodiments, the culture medium comprises arginine in an amount from about 100 mg/L to about 125 mg/L, about 105 mg/L to about 150 mg/L, about 120 mg/L to about 130 mg/L, or about 100 mg/L to about 145 mg/L of culture medium. In some embodiments, the culture medium comprises magnesium in an amount from about 0.01 mM to about 1 mM, e.g., about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, or 1 mM or any value or range therein. In some embodiments, the culture medium comprises magnesium in an amount from about 0.05 mM to about 1 mM, about 0.01 mM to about 0.78 mM, about 0.5 mM to about 1 mM, about 0.03 mM to about 0.75 mM, or about 0.25 mM to about 0.95 mM.

[0191] In some embodiments, the carbon source, chemical buffering system, one or more essential amino acids, one or more vitamins and/or cofactors, and/or one or more inorganic salts is food grade.

[0192] In some embodiments, the culture medium is lactogenic culture medium, e.g., the culture medium further comprises prolactin (e.g., mammalian prolactin, e.g., human prolactin). For example, in some embodiments, the culture medium comprises prolactin (or prolactin is added) in an amount from about 20 ng/mL to about 200 ng/L of culture medium, e.g., about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 ng/mL or any value or range therein. In some embodiments, the culture medium comprises prolactin (or prolactin is added) in an amount from about 20 ng/mL to about 195 ng/mL, about 50 ng/mL to about 150 ng/mL, about 25 ng/mL to

about 175 ng/mL, about 45 ng/mL to about 200 ng/mL, or about 75 ng/mL to about 190 ng/mL of culture medium. In some embodiments, the methods further comprise adding prolactin to the culture medium, thereby providing a lactogenic culture medium. In some embodiments, the prolactin is produced by a microbial cell and/or a human cell expressing a recombinant prolactin (e.g., a prolactin comprising a substitution of a serine residue at position 179 of the prolactin gene with aspartate (S179D), e.g., S179D-prolactin). In some embodiments, adding prolactin to the culture medium comprises conditioning culture medium by culturing cells that express and secrete prolactin, and applying the conditioned culture medium comprising prolactin to the basal surface of the monolayer of mammary cells (e.g., mammary epithelial cells, mammary myoepithelial cells and mammary progenitor cells).

[0193] In some embodiments, the culture medium further comprises other factors to improve efficiency, including, but not limited to, insulin, an epidermal growth factor, and/or a hydrocortisone. In some embodiments, the methods of the present invention further comprise adding other factors (e.g., insulin, an epidermal growth factor, and/or a hydrocortisone) to the culture medium, e.g., to improve efficiency.

Methods of Manufacturing Cultured Milk Products

[0194] Disclosed herein are methods of manufacturing a cultured milk product representing the biosynthetic output of cultured mammary epithelial cells (MECs) and plasma cells.

[0195] In some embodiments, the method comprises culturing a cell construct comprising plasma cells and mammary cells disclosed herein in a bioreactor comprising a basal compartment and an apical compartment, wherein the basal compartment comprises a culture media, and the plasma cells and mammary cells secrete the cultured milk product comprising immunoglobulins into the apical compartment.

[0196] In some embodiments, the method comprises (a) isolating mammary epithelial cells, myoepithelial cells and/or mammary progenitor cells from mammary explants from mammary tissue (e.g., breast, udder, teat tissue), biopsy sample, or raw breastmilk, to produce isolated mammary epithelial cells, myoepithelial cells and/or mammary progenitor cells; (b) isolating plasma cells from mammary tissue, biopsy sample, or raw breastmilk, to produce isolated primary plasma cells; (c) culturing the isolated mammary epithelial cells, myoepithelial cells and/or mammary progenitor cells; (d) culturing the isolated primary plasma cells; (e) depositing the cultured, isolated mammary epithelial cells, myoepithelial cells and/or mammary progenitor cells and isolated primary plasma cells on a scaffold having an upper surface and lower surface to produce a mixed population of primary plasma cells and mammary epithelial cells, mammary myoepithelial cells and mammary progenitor cells wherein the plasma cells are overlaid by the mammary epithelial cells, mammary myoepithelial cells and mammary progenitor cells; (f) cultivating the mixed population of (e) on the scaffold, to produce a monolayer of polarized mammary cells located adjacent to and above the

plasma cells, wherein the plasma cells are located adjacent to and above the upper surface of the scaffold, wherein the upper surface is located adjacent to and above the lower surface of the scaffold, and wherein the polarized mammary cells comprise an apical surface and a basal surface, thereby producing a cell construct for producing the cultured milk product comprising immunoglobulins.

[0197] In some embodiments, the method comprises: a) isolating mammary epithelial cells, myoepithelial cells, and/or mammary progenitor cells from mammary explants from mammary tissue (e.g., breast, udder, teat tissue), biopsy sample, or raw breastmilk, to produce isolated mammary epithelial cells, myoepithelial cells, and/or mammary progenitor cells; (b) isolating plasma cells from mammary tissue, biopsy sample, or raw breastmilk, to produce isolated primary plasma cells; (c) culturing the isolated mammary epithelial cells, myoepithelial cells, and/or mammary progenitor cells to produce a mixed population of primary mammary epithelial cells, mammary myoepithelial cells and mammary progenitor cells; (d) culturing the isolated primary plasma cells; (e) sorting the mixed population of mammary epithelial cells, myoepithelial cells, and/or mammary progenitor cells (e.g., selecting the primary mammary epithelial cells) to produce a population of primary mammary epithelial cells; and (f) depositing the sorted population of primary mammary epithelial cells, myoepithelial cells and/or mammary progenitor cells and isolated primary plasma cells on a scaffold having an upper surface and lower surface (g) cultivating the sorted population of mammary epithelial and plasma cells on the scaffold, to produce a monolayer of polarized primary mammary epithelial cells located adjacent to and above the plasma cells, wherein the plasma cells are located adjacent to and above the upper surface of the scaffold, wherein the upper surface is located adjacent to and above the lower surface of the scaffold, and wherein the polarized mammary cells comprise an apical surface and a basal surface, and wherein the polarized monolayer comprises an apical surface and a basal surface, thereby producing a cell construct for producing the cultured milk product comprising immunoglobulins.

[0198] In some embodiments, the method comprises (a) culturing immortalized mammary epithelial cells to produce increased numbers of immortalized mammary epithelial cells; and (b) culturing plasma cells to produce increased numbers of plasma cells; (c) cultivating the immortalized mammary epithelial cells and plasma cells of (a) and (b) on a scaffold, the scaffold having an upper surface and lower surface, to produce a monolayer of polarized immortalized mammary epithelial cells above the plasma cells on the upper surface of the scaffold, wherein the polarized monolayer comprises an apical surface and a basal surface, thereby producing a cell construct for producing the cultured milk product comprising immunoglobulins.

[0199] In certain embodiments, plasma cells are added to the culture of immortalized mammary epithelial cells. In certain embodiments, the plasma cells are cultivated with the immortalized mammary epithelial cells on the scaffold, thereby producing a cultured milk product with secretory

products of the immune cells and mammary cells (e.g., sIgA). In certain embodiments, the isolated mammary cells are immortalized prior to co-culture of the cells with the plasma cells.

[0200] Plasma cells can be derived from a primary mammary tissue sample or, e.g., from a plasma cell line. In certain embodiments, the plasma cells are stimulated to produce immunoglobins during co-culture. In certain embodiments, the plasma cells produce IgA. In certain embodiments the plasma cells produce secretory IgA. In certain embodiments, the one or more classes of immunoglobulins includes secretory IgA (sIgA). In certain embodiments, the immunoglobulins bind to an antigen of a microorganism (i.e., bacterium or virus). In certain embodiments, the immunoglobulins bind to viral or bacterial antigens capable of causing an infectious disease in humans. In certain embodiments, the immunoglobulins bind to viral or bacterial antigens that cause infections of respiratory or gastrointestinal epithelium. In certain embodiments, the immunoglobulins bind antigens from microorganisms that cause enterocolitis or sepsis in infants. In certain embodiments, plasma cells are co-cultured with MECs in a bioreactor according to methods described herein. In certain embodiments, the bioreactor is a hollow fiber bioreactor described herein.

[0201] In certain embodiments, mammary cells are modified and/or stimulated with prolactin according to the methods described herein to stimulate and optimize milk production. In certain embodiments, the mammary cells are modified to express a constitutively active prolactin receptor.

[0202] In certain embodiments, mammary epithelial cell populations are identified and isolated from primary mammary tissue samples. In some embodiments, the mammary cells are isolated and sorted via fluorescence-activated cell sorting, magnetic-activated cell sorting, and/or microfluidic cell sorting. In certain embodiments, myoepithelial mammary cells and luminal epithelial mammary cells are isolated by FACS analysis. In certain embodiments, progenitor myoepithelial mammary cells and/or progenitor luminal epithelial mammary cells are isolated by FACS analysis. Any suitable method known in the art for sorting mammary epithelial cells (e.g., luminal epithelial cells), myoepithelial cells, progenitor cells, and immune cells can be used. For example, mammary cells can be sorted using CD24, EPCAM and/or CD49f, cell surface markers.

[0203] In some embodiments, the plasma cells are isolated and sorted via fluorescence-activated cell sorting, magnetic-activated cell sorting, and/or microfluidic cell sorting. In certain embodiments, the plasma cells are sorted by FACS analysis using markers known in the art for identifying the plasma cells. In certain embodiment, plasma cells, plasma blasts or pre-plasmablasts are sorted and isolated by FACS analysis using markers known in the art (e.g., CD20, CD38, CD138, and/or CD19).

[0204] In some embodiments, the cell construct comprises a scaffold comprising an upper surface and a lower surface and a continuous monolayer of polarized mammary epithelial cells, a continuous monolayer of a polarized, mixed population of mammary epithelial cells, mammary myoepithelial cells and mammary progenitor cells, and/or a continuous monolayer of polarized immortalized

mammary epithelial cells, wherein the continuous monolayer is located on the upper surface of scaffold.

[0205] In some embodiments, the lower surface of the scaffold is adjacent to the basal compartment. In some embodiments, the apical surface of the continuous monolayer is adjacent to the apical compartment. In some embodiments, the continuous monolayer secretes milk and sIgA or IgA through its apical surface into the apical compartment, thereby producing milk comprising IgA and/or sIgA in culture.

[0206] In some embodiments, the monolayer of mammary cells forms a barrier that divides the apical compartment and the basal compartment, wherein the basal surface of the mammary cells is attached to the scaffold and the apical surface is oriented toward the apical compartment.

[0207] In some embodiments, the basal compartment is adjacent to the lower surface of the scaffold. In some embodiments, the basal compartment comprises a culture medium in fluidic contact with the basal surface of the monolayer of mammary epithelial cells (e.g., the polarized monolayer of mammary epithelial cells, the polarized the monolayer of the mixed population of mammary cells, or the polarized monolayer of immortalized mammary epithelial cells).

[0208] In some embodiments, the culture medium comprises a carbon source, a chemical buffering system, one or more essential amino acids, one or more vitamins and/or cofactors, and one or more inorganic salts.

[0209] In some embodiments, the bioreactor comprises an apical compartment that is adjacent to the apical surface of the monolayer. In some embodiments, the apical compartment is adjacent to the upper surface of the scaffold.

[0210] In some embodiments, the total cell density of mammary cells in the bioreactor is at least 10^{11} mammary cells. In some embodiments, the total cell density of mammary cells in the bioreactor is at least 10^{12} mammary cells. In some embodiments, the total cell density of mammary cells in the bioreactor is at least 10^{13} mammary cells.

[0211] In some embodiments, the total cell density of mammary cells in the bioreactor is about 20 to 55 cells per $100 \mu\text{m}^2$. In some embodiments, the total cell density of mammary cells in the bioreactor is about 20 cells per $100 \mu\text{m}^2$. In some embodiments the total cell density of mammary cells in the bioreactor is 25 cells per $100 \mu\text{m}^2$. In some embodiments, the total cell density of mammary cells in the bioreactor is about 30 cells per $100 \mu\text{m}^2$. In some embodiments, the total cell density of mammary cells in the bioreactor is about 35 cells per $100 \mu\text{m}^2$. In some embodiments, the total cell density of mammary cells in the bioreactor is about 40 cells per $100 \mu\text{m}^2$. In some embodiments, the total cell density of mammary cells in the bioreactor is about 45 cells per $100 \mu\text{m}^2$. In some embodiments, the total cell density of mammary cells in the bioreactor is about 50 cells per $100 \mu\text{m}^2$.

In some embodiments, the total cell density of mammary cells in the bioreactor is about 55 cells per $100 \mu\text{m}^2$.

[0212] In some embodiments, the total cell density of plasma cells in the bioreactor is about 200 to 500 plasma cells per mm^2 . In some embodiments, the total cell density of plasma cells in the bioreactor is about 200 plasma cells per mm^2 . In some embodiments, the total cell density of plasma cells in the bioreactor is about 300 plasma cells per mm^2 . In some embodiments, the total cell density of plasma cells in the bioreactor is about 400 plasma cells per mm^2 . In some embodiments, the total cell density of plasma cells in the bioreactor is about 500 plasma cells per mm^2 .

[0213] In some embodiments, the total surface area of mammary cells within the bioreactor is at least about 1.5 m^2 . In some embodiments, the total surface area of mammary cells within the bioreactor is at least about 2 m^2 . In some embodiments, the total surface area of mammary cells within the bioreactor is at least about 2.5 m^2 . In some embodiments, the total surface area of mammary cells within the bioreactor is at least about 3 m^2 . In some embodiments, the total surface area of mammary cells within the bioreactor is at least about 4 m^2 . In some embodiments, the total surface area of mammary cells within the bioreactor is at least about 5 m^2 . In some embodiments, the total surface area of mammary cells within the bioreactor is at least about 10 m^2 . In some embodiments, the total surface area of mammary cells within the bioreactor is at least about 15 m^2 . In some embodiments, the total surface area of mammary cells within the bioreactor is at least about 20 m^2 . In some embodiments, the total surface area of mammary cells within the bioreactor is at least about 25 m^2 . In some embodiments, the total surface area of mammary cells within the bioreactor is at least about 50 m^2 . In some embodiments, the total surface area of mammary cells within the bioreactor is at least about 100 m^2 . In some embodiments, the total surface area of mammary cells within the bioreactor is at least about 250 m^2 . In some embodiments, the total surface area of mammary cells within the bioreactor is at least about 500 m^2 .

[0214] In some embodiments, the bioreactor maintains a temperature of about 27°C to about 39°C (e.g., a temperature of about 27°C , 28°C , 29°C , 30°C , 31°C , 32°C , 33°C , 34°C , 35°C , 35.5°C , 36°C , 36.5°C , 37°C , 37.5°C , 38°C , 38.5°C or about 39°C , or any value or range therein, e.g., about 27°C to about 38°C , about 36°C to about 39°C , about 36.5°C to about 39°C , about 36.5°C to about 37.5°C , or about 36.5°C to about 38°C). In some embodiments, the bioreactor maintains a temperature of about 37°C .

[0215] In some embodiments, the bioreactor has an atmospheric concentration of CO_2 of about 4% to about 6%, e.g., an atmospheric concentration of CO_2 of about 4%, 4.25%, 4.5%, 4.75%, 5%, 5.25%, 5.5%, 5.75%, or 6% or any value or range therein, e.g., about 4% to about 5.5%, about 4.5% to about 6%, about 4.5% to about 5.5%, or about 5% to about 6%). In some embodiments, the bioreactor has an atmospheric concentration of CO_2 of about 5%.

[0216] In some embodiments, the bioreactor has an atmospheric concentration of CO₂ of about 4% to about 6%, e.g., an atmospheric concentration of CO₂ of about 4%, 4.25%, 4.5%, 4.75%, 5%, 5.25%, 5.5%, 5.75%, or 6% or any value or range therein, e.g., about 4% to about 5.5%, about 4.5% to about 6%, about 4.5% to about 5.5%, or about 5% to about 6%). In some embodiments, the bioreactor has an atmospheric concentration of CO₂ of about 5%.

[0217] In some embodiments, the method comprises monitoring the concentration of dissolved O₂ and CO₂. In some embodiments, the concentration of dissolved O₂ is maintained between about 10% to about 25% or any value or range therein (e.g., about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25%). For example, in some embodiments, the concentration of dissolved O₂ is maintained between about 12% to about 25%, about 15% to about 22%, about 10% to about 20%, about 15%, about 20%, or about 22%. In some embodiments, the concentration of CO₂ is maintained between about 4% to about 6%, e.g., a concentration of CO₂ of about 4%, 4.25%, 4.5%, 4.75%, 5%, 5.25%, 5.5%, 5.75%, or 6% or any value or range therein, e.g., about 4% to about 5.5%, about 4.5% to about 6%, about 4.5% to about 5.5%, or about 5% to about 6%). In some embodiments, the concentration of CO₂ is maintained at about 5%.

[0218] In some embodiments, the culture medium is exchanged about every day to about every 10 days (e.g., every 1 day, every 2 days, every 3 days, every 4 days, every 5 days, every 6 days, every 7 days, every 8 days, every 9 days, every 10 days, or any value or range therein, e.g., about every day to every 3 days, about every 3 days to every 10 days, about every 2 days to every 5 days). In some embodiments, the culture medium is exchanged about every day to about every few hours to about every 10 days, e.g., about every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours to about every 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 days or any value or range therein. For example, in some embodiments, the culture medium is exchanged about every 12 hours to about every 10 days, about every 10 hours to about every 5 days, or about every 5 hours to about every 3 days.

[0219] In some embodiments, the method comprises monitoring the glucose concentration and/or rate of glucose consumption in the culture medium and/or in the lactogenic culture medium. In some embodiments, the prolactin is added when the rate of glucose consumption in the culture medium is steady state.

[0220] In some embodiments, the method further comprises applying transepithelial electrical resistance (TEER) to measure the maintenance of the monolayer of epithelial cells. TEER measures a voltage difference between the fluids (e.g., media) in two compartments (e.g., between the apical and basal compartments), wherein if the barrier between the compartments loses integrity, the fluids in the two compartments may mix. When there is fluid mixing, the voltage difference will be reduced or eliminated; a voltage difference indicates that the barrier is intact. In some embodiments, upon detection of a loss of voltage by TEER, a scaffold (e.g., a Transwell® filter, a microstructured

bioreactor, a decellularized tissue, a hollow fiber bioreactor, etc.) is reinoculated with additional cells and allowed time to reestablish a barrier (e.g., a monolayer) before resuming production of the cultured milk product (e.g., milk production).

[0221] In some embodiments, the method further comprises collecting the cultured milk product from the apical compartment to produce collected cultured milk product. In some embodiments, the collecting is via a port, via gravity, and/or via a vacuum. In some embodiments, a vacuum is attached to a port.

[0222] In some embodiments, the method further comprises freezing the collected cultured milk product to produce frozen cultured milk product and/or lyophilizing the collected cultured milk product to produce lyophilized cultured milk product.

[0223] In some embodiments, the method further comprises packaging the collected cultured milk product, the frozen cultured milk product and/or the lyophilized cultured milk product into a container.

[0224] In some embodiments, the method further comprises extracting one or more components from the collected cultured milk product. Non-limiting examples of components from the collected cultured milk product include milk protein, lipid, carbohydrate, vitamin, and/or mineral contents. In some embodiments, the components from the collected cultured milk product are lyophilized and/or concentrated to produce a lyophilized or a concentrated cultured milk product component product. In some embodiments, the components from the collected cultured milk product are concentrated by, e.g., membrane filtration and/or reverse osmosis. In some embodiments, the lyophilized or concentrated cultured milk product component product is packaged in a container, optionally wherein the container is sterile and/or a food grade container. In some embodiments, the container is vacuum-sealed. In some embodiments, the container is a canister, ajar, a bottle, a bag, a box, or a pouch. In some embodiments, the cultured milk product is a standardized, sterile cultured milk product. In some embodiments, the cultured milk product is for nutritional use.

[0225] In some embodiments, the cultured milk product is produced by any method disclosed herein.

Excluded Components

[0226] By virtue of the fact that the cultured milk products produced by the methods disclosed herein are manufactured outside of the human body, the cultured milk products are free of or substantially free of certain components found in naturally-occurring breast milk. In some cases, the components are harmful (e.g., environmental contaminants and allergens) and in other cases, the naturally occurring components are simply present due to the naturally occurring milk being naturally produced. Further, the design of the live cell construct and the bioreactor enables the

production of cultured milk products disclosed herein that are free of or substantially free of cell culture media and components thereof.

Cell Culture Media

[0227] Certain components of cell culture media may pass into the cultured milk product. For example, serum albumin, linoleic acid and alpha-linolenic acid may be actively secreted by the hMECs into the apical compartment with components of the cultured milk product produced by the hMECs. In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises about 1% w/w to about 20% of cell media components greater than 150kDa. In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises about 5% w/w to about 10% of cell media components greater than 150kDa. In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises about 5% w/w of cell media components greater than 150kDa. In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises about 20% w/w to about 40% of cell media components less than 150kDa. In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises about 25% w/w to about 35% of cell media components less than 150kDa. In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises about 25% w/w to about 30% of cell media components less than 150kDa. In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises about 25% w/w of cell media components less than 150kDa.

[0228] In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises less than 80% w/w of cell media components greater than 150kDa. In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises less than 85% w/w of cell media components greater than 150kDa. In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises less than 90% w/w of cell media components greater than 150kDa. In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises less than 95% w/w of cell media components greater than 150kDa. In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises less than 97% w/w of cell media components greater than 150kDa. In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises less than 98% w/w of cell media components greater than 150kDa. In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises less than 99% w/w of cell media components greater than 150kDa.

[0229] In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises less than 60% w/w of cell media components less than 150kDa. In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises less than 65% w/w of cell media components less than 150kDa. In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises less than 70% w/w of cell media components less than 150kDa.

In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises less than 75% w/w of cell media components less than 150kDa. In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises less than 77% w/w of cell media components less than 150kDa. In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises less than 78% w/w of cell media components less than 150kDa. In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises less than 79% w/w of cell media components less than 150kDa. In some embodiments, the total dry mass of the cultured milk product disclosed herein comprises less than 80% w/w of cell media components less than 150kDa.

Environmental Contaminants and Drugs

[0230] Breast milk contains low but measurable concentrations of environmental contaminants, health-harming chemicals from industry and manufacturing products that are widely spread in the environment. Environmental contaminants are partly secreted in breast milk. The contaminant levels in breast milk reflect those in the mother's body and are therefore ideal for monitoring exposure levels. Toxic environmental contaminants can be transferred from mother to infant via breastfeeding. Persistent organic pollutants (POPs) are a family of lipophilic stable chemicals that bioaccumulate in adipose tissue and create a lasting toxic body burden. Breastfeeding provides a significant source of exposure to POPs early in human life, the effects of which are unknown.

[0231] In some embodiments, the cultured milk product does not comprise or is substantially free of one or more environmental contaminants. In some embodiments, the cultured milk product does not comprise or is substantially free of persistent organic pollutants (POPs). In some embodiments, 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 such as DDT.

[0232] Heavy metals such as mercury, lead, arsenic, cadmium, nickel, chromium, cobalt, zinc, and other potentially toxic metals that are dispersed throughout the environment also have bioaccumulative features known to accumulate in human milk and thus are of concern to the nursing infant. Metal in breast milk originates from exogenous sources, i.e., uptake via contaminated air, food, and drinking water, and endogenous release along with essential trace elements. For example, lead and mercury are equally dispersed in the human food chain, and their impact on fetal development is heavily determined by the mother's diet and nutritional status. The exposures to toxic metals have significant public health implication, even at small concentrations and acute exposures, these metals remain toxic to humans. A nursing infant may be exposed to toxic metals in a period of highest susceptibility. Nursing infants may be exposed to heavy metals through breast milk in excess of what they should, and exposure may have health implication for the infants. For

infants in particular, these exposures may have adverse effect on the developing central nervous system, leaving a life-long defect on their cognitive abilities.

[0233] Breastmilk may contain residues of any pharmaceutical or recreational drugs used by the mother. Certain prescription drugs such as chemotherapeutics are counter-indicated for breastfeeding due to the risks for the infant. Commonly used recreational intoxicants such as cannabis and alcohol transfer to breastmilk. Furthermore, psychoactive substances are detected in breastmilk from women who use these drugs while lactating, and metabolites of illicit drugs have been found in hair samples from children who were breastfed by mothers who ingested these substances while breastfeeding.

[0234] In some embodiments, the cultured milk product does not comprise or is substantially free of one or more heavy metals, such as arsenic, lead, cadmium, nickel, mercury, chromium, cobalt, and zinc. In some embodiments, the cultured milk product does not comprise or is substantially free of arsenic. In some embodiments, the cultured milk product does not comprise or is substantially free of lead. In some embodiments, the cultured milk product does not comprise or is substantially free of cadmium. In some embodiments, the cultured milk product does not comprise or is substantially free of nickel. In some embodiments, the cultured milk product does not comprise or is substantially free of mercury. In some embodiments, the cultured milk product does not comprise or is substantially free of chromium. In some embodiments, the cultured milk product does not comprise or is substantially free of cobalt. In some embodiments, the cultured milk product does not comprise or is substantially free of zinc. In some embodiments, the cultured milk product does not comprise or is substantially free of arsenic, lead, cadmium, nickel, mercury, chromium, cobalt, and zinc.

[0235] In some embodiments, the cultured milk product does not comprise or is substantially free of drugs from the group consisting of: chemotherapeutics, antidepressants, anti-anxiety medications, benzodiazepines, alcohol, cannabis, opioids, phencyclidine, cocaine, or metabolites thereof. In some embodiments, the cultured milk product does not comprise or is substantially free of drugs from the group consisting of: lithium, carbamazepine, chlorpromazine, and clozapine.

Allergens

[0236] Foreign allergenic proteins can be difficult to distinguish from endogenous human milk proteins. Food proteins with allergenic potential that have been detected in human milk include hen's egg and peanut proteins. There are eight major food allergens, known as the big 8, that are responsible for most of the serious food allergy reactions in the U.S. The big 8 list is comprised of milk, egg, fish, crustacean shellfish, tree nuts, peanuts, wheat, and soybean allergens. Proteins known to cause egg allergy include ovomucoid, ovalbumin, and conalbumin. Peanuts proteins include arachin 6, arachin 3, conarachin, main allergen Arah1, and arachin Arah2. As an example of maternal dietary protein transportation to milk, it has been shown that the consumption of one egg per day leads to higher concentrations of the chicken egg allergen ovalbumin (OVA) in human milk compared to egg-avoiding mothers.

[0237] In some embodiments, the cultured milk product does not comprise or is substantially free of one or more food allergens. In some embodiments, the cultured milk product does not comprise or is substantially free of egg, fish, crustacean shellfish, tree nuts, peanuts, wheat, and soybean allergens. In some embodiments, the cultured milk product does not comprise or is substantially free of egg allergens. In some embodiments, the cultured milk product does not comprise or is substantially free of fish allergens. In some embodiments, the cultured milk product does not comprise or is substantially free of crustacean allergens. In some embodiments, the cultured milk product does not comprise or is substantially free of tree nut allergens. In some embodiments, the cultured milk product does not comprise or is substantially free of peanut allergens. In some embodiments, the cultured milk product does not comprise or is substantially free of wheat allergens. In some embodiments, the cultured milk product does not comprise or is substantially free of soybean allergens.

[0238] In some embodiments, the cultured milk product does not comprise or is substantially free of arachin 6, arachin 3, conarachin, Arahl, and Arah2.

[0239] In some embodiments, the cultured milk product does not comprise or is substantially free of ovalbumin (OVA).

Cells

[0240] Naturally occurring breastmilk contains a substantial population of living cells representing a diversity of cell types, including human stem cells, human immune cells, and bacterial cells. Breast milk contains approximately 146,000 cells/ml and that the amount decreases in transitional (8–12 day postpartum) and mature milk (26–30 day postpartum) to 27,500 and 23,650 cells/ml, respectively. Cells include the myeloid precursors (9–20%), neutrophils (12–27%), immature granulocytes (8–17%), and non-cytotoxic T cells (6–7%). The existence of the human milk microbiome was discovered only a decade ago. It is estimated that an infant feeding on 800 ml of breast milk per day could ingest 10^7 – 10^8 bacterial cells daily. The most frequent bacteria found in human milk are those belonging to the species *Staphylococcus*, *Acinetobacter*, *Streptococcus*, *Pseudomonas*, *Lactococcus*, *Enterococcus* and *Lactobacillus*.

[0241] In some embodiments, the cultured milk product does not comprise or is substantially free of a cell. In some embodiments, the cultured milk product comprises a human mammary epithelial cell and/or a plasma cell. In some embodiments, the cultured milk product does not comprise or is substantially free of a cell other than one or more human mammary epithelial cells and/or one or more plasma cells. In some embodiments, the cultured milk product does not comprise or is substantially free of cells selected from the group consisting of: stem cells, myoepithelial cells, myeloid precursor cells, neutrophils, granulocytes, T cells, *Staphylococcus*, *Acinetobacter*, *Streptococcus*, *Pseudomonas*, *Lactococcus*, *Enterococcus* and *Lactobacillus*. In some embodiments, the cultured milk product comprises a human mammary epithelial cell and/or a plasma cell. In some

embodiments, the cultured milk product does not comprise or is substantially free of a cell other than one or more human mammary epithelial cells and/or one or more plasma cells.

Viruses

[0242] Naturally occurring breastmilk may transmit certain infectious agents, such as hepatitis B virus, hepatitis C virus, cytomegalovirus, West Nile virus, human T-cell lymphotropic virus, and human immunodeficiency virus. In some embodiments, the cultured milk product does not comprise or is substantially free of a virus. In some embodiments, the cultured milk product does not comprise or is substantially free of a virus selected from the group consisting of: hepatitis B virus, hepatitis C virus, cytomegalovirus, West Nile virus, human T-cell lymphotropic virus, and human immunodeficiency virus.

Hormones

[0243] Naturally occurring breastmilk may include hormones from maternal circulation, such as leptin, ghrelin, and adiponectin, that are synthesized outside the mammary gland and transported into milk across the mammary epithelium. In some embodiments, the cultured milk product does not comprise a hormone. In some embodiments, the cultured milk product does not comprise a hormone selected from the group consisting of: leptin, ghrelin, adiponectin, thyroxine (T4), triiodothyronine (T3) thyroid-stimulating hormone (TSH), epidermal growth factor, beta-endorphin, relaxin, cortisol, and erythropoietin.

Methods of Use

[0244] Disclosed herein, in certain embodiments, are methods of treating and/or preventing a microbial infection in a subject in need thereof, comprising administration of a cultured milk product comprising IgA or sIgA described herein, or and immunotherapeutic composition comprising isolated IgA or sIgA described herein.

[0245] In some embodiments, the subject is a human. In some embodiments, the human subject is an infant. In some embodiments, the human subject is immuno-compromised. In some embodiments, the human subject has a disease selected from: severe combined immunodeficiency (SCID), HIV/AIDS, a cancer, or an autoimmune disease. In some embodiments, the human subject has lupus or diabetes (for example, Type I diabetes or Type II diabetes). In some embodiments, the human subject is an organ or bone marrow transplant recipient. In some embodiments, the human subject is malnourished. In some embodiments, the human subject has malabsorption syndrome. In some embodiments, the human subject has wasting syndrome. In some embodiments, the human subject is geriatric. In some embodiments, the human subject has cystic fibrosis, COPD, or non-CF bronchiectasis.

[0246] In certain embodiments, the immunotherapeutic compositions and cultured milk products comprising IgA and/or sIgA are administered to patients in an effective amount for the treatment or prevention of a microbial infection. In some embodiments, the microbial infection is a bacterial infection, fungal infection or parasitic infection.

[0247] In certain embodiments, the microbial infection is a bacterial infection. Non-limiting examples of bacterial infections that can be treated and/or prevented include infections caused by: *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella*, *Shigella*, *Campylobacter*, *Staphylococcus aureus*, *Helicobacter pylori*, *E. coli*, *C. difficile*, or *Vibrio cholerae*.

[0248] In certain embodiments, the microbial infection is a viral infection. Non-limiting examples of viral infection that can be treated and/or prevented include infections caused by: influenza virus, parainfluenza virus, respiratory syncytial virus, rhinovirus, and human metapneumovirus.

[0249] In some embodiments, the microbial infection is a parasitic infection. In some embodiments, the parasitic infection is an infection of *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium* spp., or *Cystoisospora belli*.

[0250] In some embodiments, the microbial infection is a fungal infection. In some embodiments, the fungal infection is an infection of *Aspergillus*, *Cryptococcus*, or *Pneumocystis jirovecii*.

[0251] In certain embodiments, the cultured milk product immune comprising IgA or sIgA, or therapeutic composition comprising IgA or sIgA are administered orally to a subject to treat or prevent a gastrointestinal infection. In certain embodiments, the gastrointestinal infection is bacterial or viral gastroenteritis. In certain embodiments, the subject has an ulcer caused by an infection of *Helicobacter pylori*.

[0252] In some embodiments, the microbial infection is Candidiasis.

[0253] In some embodiments, the microbial infection is a respiratory infection. In some embodiments, the respiratory infection is pneumonia, bronchitis, Aspergillosis, or Cryptococcosis. In some embodiments, the respiratory infection is an infection by *B. cepacia*, *P. aeruginosa* *S. aureus*, *Aspergillus*, *Cryptococcus*, or *Pneumocystis*.

[0254] In certain embodiments, the immunotherapeutic compositions disclosed herein are administered to a subject as a nasal inhalant to treat or prevent a respiratory infection. In some embodiments, the composition is formulated for inhalation. In some embodiments, the composition is a powder. In some embodiments, the composition is formulation for administration by a nebulizer

[0255] In certain embodiments, the immunotherapeutic compositions and/or cultured milk products are administered in combination with another anti-microbial agent (i.e., antibiotic or anti-viral

agent). In certain embodiments, the cultured milk product described herein are administered with an additional nutritional product for treatment and/or prevention of a gastrointestinal infection.

[0256] Having described the present disclosure, the same will be explained in greater detail in the following examples, which are included herein for illustration purposes only, and which are not intended to be limiting to the disclosure.

EXAMPLES

[0257] The Examples that follow are illustrative of specific embodiments of the disclosure, and various uses thereof. They are set forth for explanatory purposes only and should not be construed as limiting the scope of the disclosure in any way.

EXAMPLE 1: Production of Milk Components from Human Mammary Epithelium

[0258] In this example, human mammary epithelium is recapitulated and milk is produced *in vitro*. The system and process described is exemplary and can be scaled to produce multiliter volumes of milk components. Mammary epithelial cells are expected to form a polarized monolayer on bioreactor fibers that have been precoated with one or more of laminin and collagen or other extracellular matrix proteins, as well as on uncoated fibers. When confluent, the monolayer forms a barrier that divides the intra- and extracapillary space (ECS), with the basal surface attached to the fibers and the apical surface oriented toward the ECS. Milk component production is stimulated by addition of prolactin to the media. The secreted milk components are collected from the ECS and submitted for downstream analyses of the protein, lipid, and carbohydrate content in comparison to human breast milk produced *in vivo*.

[0259] Materials for use in this Example are shown in Table 3.

Table 3. Materials

Item	Supplier	Catalog Number
Primary mammary epithelial cells	ATCC	PCS-600-010
Mammary epithelial cell basal medium	ATCC	PCS-600-30
Mammary epithelial cell growth kit	ATCC	PCS-600-040
Dulbecco's phosphate buffered saline (D-PBS)	ATCC	ATCC 30-2200
Trypsin-EDTA	ATCC	PCS-999-003
Trypsin Neutralizing Solution	ATCC	PCS-999-004
EHS (Laminin-1/111)	Sigma	L2020-1MG
Collagen-IV	Sigma	C5533-5MG

Prolactin	Shenandoah Biotechnology	100-45-500ug
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[0260] Procedures

[0261] Expansion of primary human mammary epithelia cells (HMECs)

[0262] Human mammary epithelial cells (1 ampoule; 5×10^5 cells) are expanded into one collagen-IV-coated T300 flask (or two T175 flasks) in mammary epithelial cell medium prepared with supplements provided in the kit listed in Table 3. Once an appropriate cell number is obtained, cells are rinsed with D-PBS and collected from the plates using trypsin-EDTA. Once cells are detached, trypsin activity is halted using Trypsin Neutralizing Solution. Cells are resuspended in medium and seeded into a hollow fiber bioreactor (Fibercell Systems), prepared as described below.

[0263] Preparation of hollow fiber bioreactor (C2025D, 20 kD MWCO)

[0264] Prior to seeding, a bioreactor cartridge (Fibercell Systems) is prepared by preculturing with PBS for a minimum of 24 hours. The bioreactor cartridge is optionally pre-coated by adding about 50-100 pg of one or more of collagen I, collagen IV, laminin-111 (e.g., laminin-111 isolated from Engelbreth-Holm Swarm tumor), alpha-4, alpha-5, fibronectin, and/or entactin in 3.2 mL of PBS and allowing ultrafiltration across the fiber at room temperature overnight. The uncoated or precoated cartridge is exchanged with medium and incubated overnight at room temperature. The medium is then exchanged with the cells collected from the T300 (or T175) flask(s). The reservoir volume is no more than 125 mL. The cartridge is rotated 180 degrees after seeding the cells.

[0265] Cell growth in the bioreactor and prolactin stimulation

[0266] After seeding the bioreactor, cells are grown in mammary epithelial cell growth medium supplemented with 10-25 mL DMEM/10% CDM-HD (Dulbecco's Modified Eagle Medium; Chemically Defined Medium for High Density Cell Culture) per 100 mL medium. The proportion of DMEM/CDM-HD can be adjusted based on the rate of glucose consumption.

[0267] Before stimulation of milk secretion, the medium in the ECS is flushed and replaced with PBS. To stimulate milk component secretion, medium supplemented with 100 ng/mL prolactin is added. The lactogenic medium can also be supplemented with an elevated concentration of glucose and the essential dietary precursors for milk fatty acids, linoleic acid and α -linolenic acid. The bioreactor is maintained for 10 days with sampling as described below.

[0268] Harvesting and sample preparation

[0269] Samples, comprised of supernatant from the ECS and an equivalent volume of media from the reservoir, are collected once daily for 10 days after addition of prolactin to the media. The samples are spun in a centrifuge to collect any debris and resuspended in an equivalent volume of PBS. The supernatants from the ECS and media samples are divided into 0.5 mL aliquots in

microfuge tubes and frozen at -80°C. The pellet debris is resuspended in a volume of PBS equivalent to the original sample and frozen at -80°C. Samples are processed to determine relative concentrations of milk components produced.

[0270] Scaling for multiliter production

[0271] To scale for multiliter production, the preceding procedure with relative adjustments in reagent volumes for a larger bioreactor (e.g., Fibercell Systems cat. no. C2018) is performed.

EXAMPLE 2:

[001] A cell culture system designed for the collection of milk should support compartmentalized secretion of the product such that the milk is not exposed to the media that provides nutrients to the cells. In the body, milk-producing epithelial cells line the interior surface of the mammary gland as a continuous monolayer. The monolayer is oriented such that the basal surface is attached to an underlying basement membrane, while milk is secreted from the apical surface and stored in the luminal compartment of the gland, or alveolus, until it is removed during milking or feeding. Tight junctions along the lateral surfaces of the cells ensure a barrier between the underlying tissues and the milk located in the alveolar compartment. Therefore, *in vivo*, the tissue of the mammary gland is arranged such that milk secretion is compartmentalized, with the mammary epithelial cells themselves establishing the interface and maintaining the directional absorption of nutrients and secretion of milk.

[002] The present disclosure describes a cell culture apparatus that recapitulates the compartmentalizing capability of the mammary gland that is used to collect milk from mammary epithelial cells grown outside of the body. Such an apparatus can include a scaffold to support the proliferation of mammary cells at the interface between two compartments, such that the epithelial monolayer provides a physical boundary between the nutrient medium and the secreted milk. In addition to providing a surface for growth, the scaffold provides spatial cues that guide the polarization of the cells and ensures the directionality of absorption and secretion. This invention describes the preparation, cultivation, and stimulation of mammary epithelial cells in a compartmentalizing cell culture apparatus for the production and collection of milk for nutritional use (see e.g., **FIG. 1**).

[003] Preparation of mammary epithelial cells. Mammary epithelial cells are obtained from surgical explants of dissected mammary tissue (e.g., breast, udder, teat), biopsy sample, or raw breastmilk. Generally, after surgical dissection of the mammary tissue, any fatty or stromal tissue is manually removed under aseptic conditions, and the remaining tissue of the mammary gland is enzymatically digested with collagenase and/or hyaluronidase prepared in a chemically defined nutrient media, which should be composed of ingredients that are “generally recognized as safe” (GRAS). The sample is maintained at 37 °C with gentle agitation. After digestion, a suspension of

single cells or organoids is collected, either by centrifugation or by pouring the sample through a sterile nylon cell strainer. The cell suspension is then transferred to a tissue culture plate coated with appropriate extracellular matrix components (e.g., collagen, laminin, fibronectin).

[004] Alternatively, explant specimens can be processed into small pieces, for example by mincing with a sterile scalpel. The tissue pieces are plated onto a suitable surface such as a gelatin sponge or a plastic tissue culture plate coated with appropriate extracellular matrix.

[005] The plated cells are maintained at 37 °C in a humidified incubator with an atmosphere of 5% CO₂. During incubation, the media is exchanged about every 1 to 3 days and the cells are sub-cultured until a sufficient viable cell number is achieved for subsequent processing, which includes preparation for storage in liquid nitrogen; development of immortalized cell lines through the stable transfection of genes such as SV40, TERT, or other genes associated with senescence; isolation of mammary epithelial, myoepithelial, and stem/progenitor cell types by, for example, fluorescence-activated cell sorting; and/or introduction into a compartmentalizing tissue culture apparatus for the production and collection of milk for human consumption.

[006] **Cultivation of mammary epithelial cells for the production of milk.** Milk for nutritional use is produced by mammary epithelial cells isolated as described above and cultured in a format that supports compartmentalized secretion such that separation between the nutrient medium and the product is maintained. The system relies on the ability of mammary epithelial cells to establish a continuous monolayer with appropriate apical-basal polarity when seeded onto an appropriate scaffold positioned at the interface between the apical compartment, into which milk is secreted, and the basal compartment, through which nutrient media is provided (*see, e.g., FIG. 2*). Transwell® filters placed in tissue culture plates, as well as bioreactors based on hollow fiber or microstructured scaffolds, for example, are used to support these characteristics.

[007] Following the isolation and expansion of mammary epithelial cells, the cells are suspended in a chemically defined nutrient medium composed of food-grade components and inoculated into a culture apparatus that has been pre-coated with a mixture of extracellular matrix proteins, such as collagen, laminin, and/or fibronectin. The cell culture apparatus is any design that allows for the compartmentalized absorption of nutrients and secretion of product from a polarized, confluent, epithelial monolayer. Examples include hollow fiber and microstructured scaffold bioreactors (*see, e.g., FIGS. 3 and 4, respectively*). Alternatives include other methods of 3-dimensional tissue culture, such as the preparation of decellularized mammary gland as a scaffold, repopulated with stem cells to produce a functional organ *in vitro*, or collection of milk from the lumen of mammary epithelial cell organoids or “mammospheres” grown either in a hydrogel matrix or in suspension.

[008] The apparatus includes sealed housing that maintains a temperature of about 37°C in a humidified atmosphere of about 5% CO₂. Glucose uptake is monitored to evaluate the growth of the culture as the cells proliferate within the bioreactor. Stabilization of glucose consumption indicates

that the cells have reached a confluent, contact-inhibited state. The integrity of the monolayer is ensured using transepithelial electrical resistance. Sensors monitor concentrations of dissolved O₂ and CO₂ in the media at multiple locations. A computerized pump circulates media through the bioreactor at a rate that balances the delivery of nutrients with the removal of metabolic waste such as ammonia and lactate. Media can be recycled through the system after removal of waste using Lactate Supplementation and Adaptation technology (Freund et al. 2018 *Int J Mol Sci.* 19(2)) or by passing through a chamber of packed zeolite.

[009] Stimulation of milk production. *In vivo* and in cultured mammary epithelial cells, the production and secretion of milk is stimulated by prolactin. In culture, prolactin can be supplied exogenously in the nutrient media at concentrations approximating those observed in the body during lactation, e.g., about 20 ng/mL to about 200 ng/mL. Purified prolactin can be obtained commercially; however, alternative methods of providing prolactin or stimulating lactation are employed, including expression and purification of the recombinant protein from microbial or mammalian cell cultures. Alternatively, conditioned media prepared by culturing cells that express and secrete prolactin can be applied to mammary epithelial cell cultures to stimulate lactation. Bioreactors can be set up in series such that media passing through a culture of cells expressing prolactin or other key media supplements is conditioned prior to exposure to mammary cells grown in a compartmentalizing culture apparatus as described.

[0010] Other approaches to upregulate milk production and/or spare the use of exogenous prolactin include molecular manipulation of the signaling pathways that are regulated by binding of prolactin to its receptor on the surface of mammary epithelial cells, such as the following: (a) expression of constructs targeting the posttranslational modification of prolactin; (b) expression of alternative isoforms of the prolactin receptor; (c) expression of a chimeric prolactin receptor in which the extracellular domain is exchanged with the binding site for a different ligand; (d) introduction of a gene encoding a constitutively or conditionally active prolactin receptor or modified versions of its downstream effectors such as STAT5 or Akt; (e) knockout or modification of the PER2 circadian gene; and/or (f) molecular approaches aimed at increasing the rate of nutrient uptake at the basal surface of the mammary epithelial monolayer.

[0011] Collection of milk. Secreted milk is collected continuously or at intervals through, for example, a port installed in the apical compartment of the culture apparatus. A vacuum is applied to the port to facilitate collection and also contributes to the stimulation of further production. The collected milk is packaged into sterile containers and sealed for distribution, frozen or lyophilized for storage, or processed for the extraction of specific components.

[0012] The present invention provides mammary epithelial cell cultures for the production of milk for nutritional use. In addition to human breast milk, this method may be used to produce milk from other mammalian species, for example, for human consumption or veterinary use. Because it has not

been previously possible to produce milk outside the body, this technology may result in novel commercial opportunities, in addition to providing an alternative mode of production for existing products. The social and economic effects of the commercial development of this technology are broad and far reaching. Production of human breast milk from cultured cells may provide a means to address infant malnutrition in food-scarce communities, provide essential nutrients to premature infants who are unable to breastfeed, and offer mothers a new option for feeding their babies that provides optimal nutrition with the convenience of infant formula. Production of cow or goat milk provides an opportunity to reduce the environmental, social, and animal welfare effects of animal agriculture. The process described here addresses an important gap in the emerging field of cellular agriculture and introduces an opportunity to dramatically update the human food supply without compromising our biological and cultural attachment to the most fundamental of our nutrition sources.

EXAMPLE 3: Manufacturing milk compositions comprising immunoglobins

[0272] Primary mammary tissue is collected from a healthy donor. CD20+ immune cells and mammary epithelial cell populations are sorted by FACS analysis using known markers for the myoepithelial and luminal epithelial cell populations. The isolated mammary cells and immune cells are immortalized prior to co-culture of the cells. The immortalized immune cells are co-cultured with immortalized isolated mammary epithelial cells in a hollow fiber bioreactor and grown to confluence. The culture is stimulated with prolactin to stimulate milk production. The secretory cell culture product comprising immunoglobulins, including secretory IgA is then harvested by a non-disruptive withdrawal mechanism.

EXAMPLE 4: Manufacturing of immunotherapy compositions from co-cultured mucosal epithelial cells and immune cells

[0273] Primary mammary epithelial cells and CD20+ immune cells are harvested from tissue derived from patients who have recovered from a mucosal infection. Patients are selected that have been tested positive for serum that exhibits viral neutralization activity. The CD20+ immune cells are co-cultured with the mammary epithelial cells in a hollow fiber bioreactor. The immune cells are grown in 2-D culture, followed by inoculation into a hollow fiber bioreactor and grown to maximum density (i.e., superconfluence) within the bioreactor. The co-culture of the mucosal epithelial cells and immune cells are stimulated to produce immunoglobins including secretory IgA. The co-culture is stimulated with one or more of a stimulant selected from, a viral protein, a viral protein fragment, prolactin, TGF- β , and interleukin 10 (IL-10). The products from the co-culture of mucosal epithelial cells and stimulated immune cells are harvested using a standard vacuum method. In certain embodiments, the secreted product is purified. The immunoglobulins are purified from the harvested secreted product. The purified secreted product is formulated as an inhalant for human pulmonary administration and is effective in the treatment of respiratory infection.

EXAMPLE 5: Treatment Of Respiratory Infection With Immunotherapy Composition From Co-Cultured Mucosal Epithelial Cells And Immune Cells

[0274] An immunotherapy composition produced as described above in Example 3 is formulated as an inhalant and is administered to a patient harboring a respiratory infection, in an amount effective to treat the infection. The immunotherapy composition is effective at reducing the severity of symptoms of the infection in the patient.

EXAMPLE 6: CULTURED MILK PRODUCT

[0275] Mammary epithelial cells (MECs) produce the majority of milk components and regulate milk composition inside the body during lactation. Here we describe the composition of cell-cultured human milk products that can be obtained by culturing human MECs (hMECs) in a hollow fiber bioreactor (HFBR), which provides a permeable scaffold and allows the formation of an adherent culture that maintains an epithelial barrier between cell culture media, which is circulated through the bioreactor capillaries, and the secreted product, which is collected from the extracapillary space (ECS).

[0276] Production and Analysis

[0277] The product compositions described in this example represent the biosynthetic output of hMECs derived from a variety of sources cultured in HFBRs under lactogenic conditions (Figure 9A). The cellular inputs include publicly available primary and immortal hMECs (Table 4).

Table 4. Cellular inputs and culture media formulations for the production of human milk products.			
Cellular Input	Basal Media	Growth Media	Lactogenic Media
Primary hMEC Donor #1	Mammary Epithelial Cell Media	ATCC Mammary Epithelial Cell Growth Kit	Growth Media + 200 – 400 ng/mL prolactin
Primary hMEC Donor #2	Mammary Epithelial Cell Media (ATCC)	Basal Media + Mammary Epithelial Cell Growth Kit (ATCC)	Growth Media + 200 – 400 ng/mL prolactin (Sigma)
Immortal hMEC PMC42-LA	RPMI-1640 (Gibco)	Basal Media + 10% FBS (Sigma)	Growth Media + 200 – 800 ng/mL prolactin (Sigma)
Immortal hMEC MCF10A	MEBM (Lonza)	MEGM (Lonza)	MEGM + 200 – 400 ng/mL prolactin (Sigma)

[0278] Briefly, after expansion in tissue culture flasks, hMECs were inoculated into the ECS of an HFBR, and oxygenated culture media was circulated through the capillaries using a peristaltic pump.

[0279] Growth media to support the proliferative phase of the process corresponds to the designated Basal media, supplemented with required growth factors to sustain proliferation.

[0280] After inoculation, proliferation was monitored by evaluating the daily glucose utilization rate (GUR) with a glucometer (AccuCheck). An increasing GUR corresponds to increased metabolic activity as a result of an increased number of cells during the proliferative phase of the process. Stabilization or reduction of the GUR indicates that the cells have achieved a confluent state and growth has become contact-inhibited. Once confluence was achieved, the culture media circulating through the capillaries of the HFBR was transitioned to a Lactogenic media supplemented with recombinant human prolactin (Sigma). Notably, the transition to lactogenic media was observed to correspond to a second phase of increasing GUR associated with the elevated metabolic demands of milk biosynthesis, including the production of lactose (Figure 9B.) During the lactogenic phase of the process, the ECS was sampled at regular intervals for analytical testing by using a syringe to introduce fresh PBS into one ECS access port at the same time that an equal volume of the product sample was withdrawn through a syringe at the other ECS access port.

[0281] Under lactogenic stimulation, hMECs achieved a state of terminal differentiation, forming a confluent monolayer with apical/basal polarity and tight junctions at the lateral surfaces, resulting in an impermeable barrier between the basal compartment and the apical compartment. The basal surface attached to the extracellular matrix that was deposited over capillaries, and the apical surface oriented toward the ECS. With this orientation, the hMECs assembled their absorption and secretion machineries along the apical/basal axis, allowing nutrient uptake at the basal surface from media that was perfused through the capillaries, and product secretion at the apical surface, into the ECS, where it accumulated until it was withdrawn from the unit. Product accumulation was demonstrated by the detection of lactose, beta-casein, and increased total protein content within the ECS (Figure 9B-D).

[0282] The rate of product accumulation in the ECS was determined by the number of cells that the HFBR could accommodate, which was limited by the available surface area for cell attachment and media perfusion. This process was carried out using commercially available HFBRs that could accommodate approximately 10^8 (Fibercell Systems, C2025D) to 10^{11} (Fibercell Systems C2018) cells. We estimate that a lactating human mammary gland, representing approximately 1 kg of tissue, contains approximately 10^{11} cells, and that a HFBR capable of supporting this cell number would be able to produce a daily output similar to that of a lactating mammary gland. A theoretical yield based on these estimates is supported by quantitative analysis of the total protein content of our ECS samples collected during lactogenic stimulation (Figure 9D). Furthermore, the increased protein content of the product withdrawn from the ECS compared to the culture media confirms that the

cells provide a functional barrier between the compartments and preclude the diffusive transfer of media components into the product.

[0283] Macromolecular Product Composition

[0284] In addition to confirming that the total protein production by hMECs grown in a HFBR corresponds to that expected for milk, we undertook a series of untargeted and targeted analyses to evaluate the molecular composition of these products and ascertain the presence of human milk components. Mass spectrometry was used to investigate the protein, lipid, and carbohydrate contents of our products over time during runs spanning up to 90 days, and the top 10 most abundant human milk components from each category are presented in Table 5. Corresponding analyses of the cell culture media formulations used in each run confirm that the listed components were synthesized by the hMECs, as they were not present in the media.

Table 5. Top protein, lipid, and carbohydrate components of human milk observed in samples collected from hMECs cultured under lactogenic stimulation in a hollow fiber bioreactor, listed in order of abundance	
Fraction	Component
Protein	Beta-casein
	Alpha-lactalbumin
	Kappa-casein
	Alpha-S1-casein
	Lactoferrin
	Butrophilin
	Osteopontin
	Lactadherin
	Lysozyme
	Mucin-4
	Mucin-1
Fatty Acids	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, erucic acid
	C24:1, nervonic acid
Carbohydrate	Lactose
	Glucose
	3'-SL
	6'-SL
	LNT
	LNnT

[0285] Beyond the nutritional content, it is important to note that the detected components represent a number of the known bioactive species in breastmilk and are associated with development of the infant immune system and defense, gut development, and brain development. Of particular interest, we have also detected numerous free oxylipin metabolites of polyunsaturated fatty acids, such as 9-hydroxyoctadecadienoic acid, which have been reported to have anti-inflammatory and pro-inflammatory activity.

[0286] Conclusion

[0287] These results and our ongoing analyses support the feasibility of the production of human milk products from cultured hMECs, including key nutritional and bioactive components. The composition described here represents data pooled across products, as a commercial process may include post-production blending of products obtained from multiple units to achieve a specified composition.

[0288] The foregoing examples are illustrative of the present disclosure and are not to be construed as limiting thereof. Although the disclosure has been described in detail with reference to preferred embodiments, variations and modifications exist within the scope and spirit of the disclosure as described and defined in the following claims.