LIVE CELL CONSTRUCTS FOR PRODUCTION OF CULTURED MILK PRODUCT AND METHODS USING THE SAME

CROSS-REFERENCE

[001] This application claims the benefit of priority from U.S. Provisional Application No. 62/958,407, filed on January 8, 2020, and U.S. Provisional Application No. 63/199,164, filed on December 10, 2020, the contents of each are incorporated herein by reference in their entireties.

FIELD OF THE INVENTION

[002] This invention relates to live cell constructs and methods using the same for *in vitro* and/or *ex vivo* production of cultured milk product from cultured mammary cells.

BACKGROUND OF THE INVENTION

[003] 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.

[004] 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.

[005] The present invention overcomes shortcomings in the art by providing live cell constructs and methods using the same for *in vitro* and/or *ex vivo* production of cultured milk product from cultured mammary cells.

SUMMARY OF THE INVENTION

[005a] In one aspect, provide herein is a method of producing an isolated cultured milk product from mammary cells, the method comprising: (a) culturing a live cell construct in a bioreactor under conditions which produce the cultured milk product, said live cell construct comprising: (i) 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; (ii) a matrix material disposed on the exterior surface of the three-dimensional scaffold; (iii) a culture media disposed within the interior cavity/basal chamber and in fluidic contact with the internal surface of the three dimensional scaffold and the basal surface of the polarized mammary cells, wherein the culture media substantially does not contact the cultured milk product; (iv) a confluent monolayer of polarized mammary cells disposed on the matrix material, wherein the mammary cells are selected from the group consisting of: live primary mammary epithelial cells, live mammary myoepithelial cells, live mammary progenitor cells, and live immortalized mammary epithelial cells, and wherein the polarized mammary cells comprise an apical surface from which the cultured milk product is secreted and a basal surface; and said bioreactor comprising an apical compartment that is in fluidic contact with the apical surface of the mammary cells, is substantially isolated from the interior cavity of the live cell construct, and is substantially free of cell culture medium; and (b) isolating the cultured milk product secreted into the apical compartment from the apical surface of the mammary cells.

[005b] In one aspect, provided herein is a bioreactor, comprising: (a) an apical compartment comprising a cultured milk product; and (b) at least one live cell construct comprising: (i) 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; (ii) a matrix material disposed on the exterior surface of the three-dimensional scaffold; (iii) a culture media disposed within the interior cavity/basal chamber and in fluidic contact with the internal surface; and (iv) a confluent monolayer of polarized mammary cells

disposed on the matrix material, wherein the mammary cells are selected from the group consisting of: live primary mammary epithelial cells, live mammary myoepithelial cells, live mammary progenitor cells, live immortalized mammary epithelial cells, live immortalized mammary myoepithelial cells, and live immortalized mammary progenitor cells; and wherein the polarized mammary cells comprise an apical surface from which the cultured milk product is secreted and a basal surface;

wherein the apical surface of the mammary cells is in fluidic contact with the apical compartment.

[006] Disclosed herein, in certain embodiments, are live 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

What is claimed is:

1. A method of producing an isolated cultured milk product from mammary cells, the method comprising:

(a) culturing a live cell construct in a bioreactor under conditions which produce the cultured milk product,

said live cell construct comprising:

(i) 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;

(ii) a matrix material disposed on the exterior surface of the three-dimensional scaffold;

(iii) a culture media disposed within the interior cavity/basal chamber and in fluidic contact with the internal surface of the three dimensional scaffold and the basal surface of the polarized mammary cells, wherein the culture media substantially does not contact the cultured milk product;

(iv) a confluent monolayer of polarized mammary cells disposed on the matrix material, wherein the mammary cells are selected from the group consisting of: live primary mammary epithelial cells, live mammary myoepithelial cells, live mammary progenitor cells, and live immortalized mammary epithelial cells, and wherein the polarized mammary cells comprise an apical surface from which the cultured milk product is secreted and a basal surface; and

said bioreactor comprising an apical compartment that is in fluidic contact with the apical surface of the mammary cells, is substantially isolated from the interior cavity of the live cell construct, and is substantially free of cell culture medium; and

(b) isolating the cultured milk product secreted into the apical compartment from the apical surface of the mammary cells.

2. The method of claim 1, wherein the basal surface of the mammary cells is in fluidic contact with the culture media.

3. The method of claim 1, wherein total cell density of mammary cells within the bioreactor is at least 10^{11}.

4. The method of claim 1, wherein total surface area of mammary cells within the bioreactor is at least 1.5m^2.

5. The method of claim 1, wherein the matrix material comprises one or more extracellular matrix proteins.

6. The method of claim 1, wherein the culturing is carried out at a temperature of about 27°C to about 39°C.

7. The method of claim 1, wherein the culturing is carried out at an atmospheric concentration of CO_2 of about 4% to about 6%.

8. The method of claim 7, wherein the culturing is carried out at an atmospheric concentration of CO_2 of 5%.

9. The method of claim 1, wherein the culture medium comprises a carbon source, a chemical buffering system, one or more essential amino acids, one or more vitamins or cofactors, and one or more inorganic salts.

10. The method of claim 1, wherein the scaffold comprises a natural polymer, a biocompatible synthetic polymer, a synthetic peptide, or any combination thereof.

11. The method of claim 10, wherein the natural polymer is collagen, chitosan, cellulose, agarose, alginate, gelatin, elastin, heparan sulfate, chondroitin sulfate, keratan sulfate, and/or hyaluronic acid.

12. The method of claim 10, wherein the biocompatible synthetic polymer is polysulfone, polyvinylidene fluoride, polyethylene co-vinyl acetate, polyvinyl alcohol, sodium polyacrylate, an acrylate polymer, and/or polyethylene glycol.

13. A bioreactor, comprising:

(a) an apical compartment comprising a cultured milk product; and

(b) at least one live cell construct comprising:

(i) 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;

(ii) a matrix material disposed on the exterior surface of the three-dimensional scaffold;

(iii) a culture media disposed within the interior cavity/basal chamber and in fluidic contact with the internal surface; and

(iv) a confluent monolayer of polarized mammary cells disposed on the matrix material, wherein the mammary cells are selected from the group consisting of: live primary mammary epithelial cells, live mammary myoepithelial cells, live mammary progenitor cells, live immortalized mammary epithelial cells, live immortalized mammary myoepithelial cells, and live immortalized mammary progenitor cells; and wherein the polarized mammary cells comprise an apical surface from which the cultured milk product is secreted and a basal surface;

wherein the apical surface of the mammary cells is in fluidic contact with the apical compartment.

14. The bioreactor of claim 13, wherein the total cell density of mammary cells within the bioreactor is at least 10^{11}.

15. The bioreactor of claim 13, wherein the total surface area of mammary cells within the bioreactor is at least 1.5 m^2.