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, provided herein is a live 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;

and (d) an at least 70% 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.

[005b] 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) an at least 70% confluent monolayer of polarized mammary cells, 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; (ii) 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; (iii) a matrix material disposed on the exterior surface of the three-dimensional scaffold, wherein the at least 70% confluent monolayer of polarized cells is disposed upon the matrix material; and (iv) 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; and said bioreactor comprising an apical compartment that is in fluidic contact with the apical surface of the mammary cells and is substantially isolated from the internal cavity of the live cell construct and the cell culture media disposed within the interior cavity; and (b) isolating the cultured milk product secreted into the apical compartment from the apical surface of the mammary cells.

[005c] In one aspect, provided herein is a milk product produced by mammary cells cultured *in vitro* and/or *ex vivo,* wherein the milk product does not comprise cell culture medium, an environmental contaminant, or a food allergen.

[005d] 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) an at least 70% 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;

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