(e.g., organ-specific epithelial cells harvested from subjects, lineage-committed epithelial cells derived from pluripotent stem cells and/or differentiated epithelial cells) can be challenging to proliferate and expand in vitro and typically have a very limited lifespan in culture. To study epithelial cells in vitro or ex vivo, a form of genetic manipulation such as inserting viral or cellular oncogenes, often is required to allow the cells to survive more than a few passages. These genetic manipulations, however, change the genetic background and physiology of the cells such that these cells may not resemble or function like normal epithelial cells. Moreover, these genetically modified cells would not be candidates for implantation into an animal.

Methods of culturing and expanding epithelial cells (e.g., cells harvested from subjects, cells derived from stem cells) for extended periods of time, without genetically altering the cells, would be useful for a variety of purposes including research applications, personalized medicine applications, and transplantation.

Summary

In one aspect, there is provided a method for proliferating epithelial cells ex vivo, comprising:

expanding the number of cells in an originating epithelial cell population comprising differentiated epithelial cells under serum-free and feeder-cell free expansion culture conditions, thereby generating an expanded epithelial cell population, wherein the expansion culture conditions comprise one or more agents that inhibit transforming growth factor beta (TGF-beta) signaling in the population, and

activating telomerase reverse transcriptase in the epithelial cell population.

In another aspect, there is provided a method for genetically modifying cells, comprising contacting cells with one or more agents that introduce a genetic modification to the cells, wherein the cells have been expanded by a method comprising:

expanding the number of cells in an originating epithelial cell population comprising differentiated epithelial cells under serum-free and feeder-cell free expansion culture conditions, thereby generating an expanded epithelial cell population, wherein the expansion culture conditions comprise one or more agents that inhibit transforming growth factor beta (TGF-beta) signaling in the population, and

activating telomerase reverse transcriptase in the epithelial cell population

Provided herein in certain embodiments are methods for proliferating differentiated epithelial cells ex vivo, which method comprises a) culturing differentiated epithelial cells under serum-free and feeder-cell free conditions; and b) inhibiting TGF-beta signaling in the differentiated epithelial cells during the culturing in (a).

Also provided herein in certain embodiments are methods for proliferating formerly quiescent epithelial cells ex vivo, which method comprises a) culturing formerly quiescent epithelial cells under serum-free and feeder-cell free conditions; and b) inhibiting TGF-beta signaling in the formerly quiescent epithelial cells during the culturing in (a).

Also provided herein in certain embodiments are methods for proliferating lineage-committed epithelial cells ex vivo, which method comprises a) culturing lineage-committed epithelial cells under serum-free and feeder-cell free conditions; and b) inhibiting TGF-beta signaling in the lineage-committed epithelial cells during the culturing in (a).

Also provided herein in certain embodiments are methods for proliferating epithelial cells ex vivo, which method comprises a) culturing epithelial cells under feeder-cell free conditions; b) inhibiting TGF-beta signaling in the epithelial cells during the culturing in (a); and c) inhibiting the activity of p21-

activated kinase (PAK) in the epithelial cells during the culturing in (a). In some embodiments, the epithelial cells are differentiated epithelial cells, formerly quiescent epithelial cells and/or lineage-committed epithelial cells.

Also provided herein in certain embodiments are methods for proliferating epithelial cells ex vivo, which method comprises a) culturing epithelial cells under serum-free and feeder-cell free conditions; b) inhibiting TGF-beta signaling in the epithelial cells during the culturing in (a); and c) inhibiting the activity of myosin II in the epithelial cells during the culturing in (a). In some embodiments, the epithelial cells are differentiated epithelial cells, formerly quiescent epithelial cells and/or lineage-committed epithelial cells.

Also provided herein in certain embodiments are methods for proliferating differentiated epithelial cells ex vivo, which method comprises a) culturing differentiated epithelial cells under feeder-cell free conditions; b) activating telomerase reverse transcriptase in the differentiated epithelial cells; and c) modulating cytoskeletal structure in the differentiated epithelial cells.

Also provided herein in certain embodiments are methods for proliferating formerly quiescent epithelial cells ex vivo, which method comprises a) culturing formerly quiescent epithelial cells under feeder-cell free conditions; b) activating telomerase reverse transcriptase in the formerly quiescent epithelial cells; and c) modulating cytoskeletal structure in the formerly quiescent epithelial cells.

Also provided herein in certain embodiments are methods for proliferating lineage-committed epithelial cells ex vivo, which method comprises a) culturing lineage-committed epithelial cells under feeder-cell free conditions; b) activating telomerase reverse transcriptase in the lineage-committed epithelial cells; and c) modulating cytoskeletal structure in the lineage-committed epithelial cells.

Provided herein in certain embodiments is a serum-free cell culture medium for proliferating differentiated epithelial cells ex vivo under feeder-cell free conditions, which serum-free medium comprises one or more TGF-beta inhibitors (e.g., one or more TGF-beta signaling inhibitors). Also provided herein in certain embodiments is a serum-free cell culture medium for proliferating differentiated epithelial cells ex vivo under feeder-cell free conditions, which serum-free medium comprises a small molecule inhibitor consisting of a TGF-beta inhibitor (e.g., a TGF-beta signaling inhibitor).

Provided herein in certain embodiments is a serum-free cell culture medium for proliferating formerly quiescent epithelial cells ex vivo under feeder-cell free conditions, which serum-free medium comprises one or more TGF-beta inhibitors (e.g., one or more TGF-beta signaling inhibitors). Also provided herein in certain embodiments is a serum-free cell culture medium for proliferating formerly quiescent epithelial cells ex vivo under feeder-cell free conditions, which serum-free medium comprises a small molecule inhibitor consisting of a TGF-beta inhibitor (e.g., a TGF-beta signaling inhibitor).

Provided herein in certain embodiments is a serum-free cell culture medium for proliferating lineage-committed epithelial cells ex vivo under feeder-cell free conditions, which serum-free medium comprises one or more TGF-beta inhibitors (e.g., one or more TGF-beta signaling inhibitors). Also provided herein in certain embodiments is a serum-free cell culture medium for proliferating lineage-committed epithelial cells ex vivo under feeder-cell free conditions, which serum-free medium comprises a small molecule inhibitor consisting of a TGF-beta inhibitor (e.g., a TGF-beta signaling inhibitor).

Provided herein in certain embodiments is a serum-free cell culture medium for proliferating differentiated epithelial cells ex vivo under feeder-cell free conditions, which serum-free medium comprises one or more telomerase reverse transcriptase activators and one or more cytoskeletal structure modulators. Also provided herein in certain embodiments is a serum-free cell culture medium for proliferating differentiated epithelial cells ex vivo under feeder-cell free conditions, which serum-free medium comprises small molecules consisting of a telomerase reverse transcriptase activator and a cytoskeletal structure modulator.

Provided herein in certain embodiments is a serum-free cell culture medium for proliferating formerly quiescent epithelial cells ex vivo under feeder-cell free conditions, which serum-free medium comprises one or more telomerase reverse transcriptase activators and one or more cytoskeletal structure modulators. Also provided herein in certain embodiments is a serum-free cell culture medium for proliferating formerly quiescent epithelial cells ex vivo under feeder-cell free conditions, which serum-free medium comprises small molecules consisting of a telomerase reverse transcriptase activator and a cytoskeletal structure modulator.

Provided herein in certain embodiments is a serum-free cell culture medium for proliferating lineage-committed epithelial cells ex vivo under feeder-cell free conditions, which serum-free medium

comprises one or more telomerase reverse transcriptase activators and one or more cytoskeletal structure modulators. Also provided herein in certain embodiments is a serum-free cell culture medium for proliferating lineage-committed epithelial cells ex vivo under feeder-cell free conditions, which serum-free medium comprises small molecules consisting of a telomerase reverse transcriptase activator and a cytoskeletal structure modulator.

Also provided herein in certain embodiments is a cell culture medium for proliferating epithelial cells ex vivo under feeder-cell free conditions, which medium comprises one or more TGF-beta inhibitors (e.g., one or more TGF-beta signaling inhibitors) and one or more PAK1 inhibitors. Also provided herein in certain embodiments is a cell culture medium for proliferating epithelial cells ex vivo under feeder-cell free conditions, which medium comprises small molecules (e.g., small molecule inhibitors) consisting of a TGF-beta inhibitor (e.g., a TGF-beta signaling inhibitor) and a PAK1 inhibitor. In some embodiments, the epithelial cells are differentiated epithelial cells, formerly quiescent epithelial cells and/or lineage-committed epithelial cells.

Also provided herein in certain embodiments is a cell culture medium for proliferating epithelial cells ex vivo under feeder-cell free conditions, which medium comprises one or more TGF-beta inhibitors (e.g., one or more TGF-beta signaling inhibitors) and one or more myosin II inhibitors. Also provided herein in certain embodiments is a cell culture medium for proliferating epithelial cells ex vivo under feeder-cell free conditions, which medium comprises small molecules (e.g., small molecule inhibitors) consisting of a TGF-beta inhibitor (e.g., a TGF-beta signaling inhibitor) and a myosin II inhibitor. In some embodiments, the epithelial cells are differentiated epithelial cells, formerly quiescent epithelial cells and/or lineage-committed epithelial cells.

Provided herein in certain embodiments is a population of ex vivo proliferated (e.g., expanded) epithelial cells produced by a method comprising a) culturing and proliferating differentiated epithelial cells under serum-free and feeder-cell free conditions; and b) inhibiting TGF-beta signaling in the differentiated epithelial cells during the culturing and proliferating in (a).

Provided herein in certain embodiments is a population of ex vivo proliferated (e.g., expanded) epithelial cells produced by a method comprising a) culturing and proliferating formerly quiescent epithelial cells under serum-free and feeder-cell free conditions; and b) inhibiting TGF-beta signaling in the formerly quiescent epithelial cells during the culturing and proliferating in (a).

Provided herein in certain embodiments is a population of ex vivo proliferated (e.g., expanded) epithelial cells produced by a method comprising a) culturing and proliferating lineage-committed epithelial cells under serum-free and feeder-cell free conditions; and b) inhibiting TGF-beta signaling in the lineage-committed epithelial cells during the culturing and proliferating in (a).

Also provided herein in certain embodiments is a population of ex vivo proliferated (e.g., expanded) epithelial cells produced by a method comprising a) culturing and proliferating epithelial cells under feeder-cell free conditions; b) inhibiting TGF-beta signaling in the epithelial cells during the culturing and proliferating in (a); and c) inhibiting the activity of p21-activated kinase (PAK) in the epithelial cells during the culturing and proliferating in (a). In some embodiments, the epithelial cells are differentiated epithelial cells, formerly quiescent epithelial cells and/or lineage-committed epithelial cells.

Also provided herein in certain embodiments is a population of ex vivo proliferated (e.g., expanded) epithelial cells produced by a method comprising a) culturing and proliferating epithelial cells under serum-free and feeder-cell free conditions; b) inhibiting TGF-beta signaling in the epithelial cells during the culturing and proliferating in (a); and c) inhibiting the activity of myosin II in the epithelial cells during the culturing and proliferating in (a). In some embodiments, the epithelial cells are differentiated epithelial cells, formerly quiescent epithelial cells and/or lineage-committed epithelial cells.

Provided herein in certain embodiments is a population of ex vivo proliferated (e.g., expanded) epithelial cells produced by a method comprising a) culturing and proliferating differentiated epithelial cells under serum-free and feeder-cell free conditions; b) activating telomerase reverse transcriptase in the differentiated epithelial cells during the culturing and proliferating in (a); and c) modulating cytoskeletal structure in the differentiated epithelial cells during the culturing and proliferating in (a).

Provided herein in certain embodiments is a population of ex vivo proliferated (e.g., expanded) epithelial cells produced by a method comprising a) culturing and proliferating formerly quiescent epithelial cells under serum-free and feeder-cell free conditions; b) activating telomerase reverse transcriptase in the formerly quiescent epithelial cells during the culturing and proliferating in (a); and c) modulating cytoskeletal structure in the formerly quiescent epithelial cells during the culturing and proliferating in (a).

Provided herein in certain embodiments is a population of ex vivo proliferated (e.g., expanded) epithelial cells produced by a method comprising a) culturing and proliferating lineage-committed epithelial cells under serum-free and feeder-cell free conditions; b) activating telomerase reverse transcriptase in the lineage-committed epithelial cells during the culturing and proliferating in (a); and c) modulating cytoskeletal structure in the lineage-committed epithelial cells during the culturing and proliferating in (a).

Also provided herein in certain embodiments are cell culture compositions comprising a defined serum-free cell culture medium, a lipids mix, EGF, FGF, albumin, a TGF-beta inhibitor (e.g., a TGF-beta signaling inhibitor), and a Rho kinase inhibitor (e.g., a Rho-associated protein kinase inhibitor).

Also provided herein in certain embodiments are cell culture compositions consisting of a defined serum-free cell culture medium, a lipids mix, EGF, FGF, albumin, a TGF-beta inhibitor (e.g., a TGF-beta signaling inhibitor), and a Rho kinase inhibitor (e.g., a Rho-associated protein kinase inhibitor).

Also provided herein in certain embodiments are cell culture compositions comprising a defined serum-free cell culture medium, a lipids mix, EGF, FGF, albumin, a TGF-beta inhibitor (e.g., a TGF-beta signaling inhibitor), a Rho kinase inhibitor (e.g., a Rho-associated protein kinase inhibitor), and a beta-adrenergic agonist (e.g., a beta-adrenergic receptor agonist).

Also provided herein in certain embodiments are cell culture compositions consisting of a defined serum-free cell culture medium, a lipids mix, EGF, FGF, albumin, a TGF-beta inhibitor (e.g., a TGF-beta signaling inhibitor), a Rho kinase inhibitor (e.g., a Rho-associated protein kinase inhibitor), and a beta-adrenergic agonist (e.g., a beta-adrenergic receptor agonist).

Also provided herein in certain embodiments are methods for proliferating epithelial cells ex vivo, comprising expanding the number of cells in an originating epithelial cell population derived from differentiated tissue under feeder-cell free expansion culture conditions, thereby generating an expanded epithelial cell population, where the expansion culture conditions comprise an agent that activates telomerase reverse transcriptase in the population and/or inhibits transforming growth factor beta (TGF-beta) signaling in the population; the originating epithelial cell population is capable of 25 population doublings or more when cultured under the expansion culture conditions; and the originating epithelial cell population is capable of no more than 20 population doublings when cultured under control culture conditions that do not include the agent.

Also provided herein in certain embodiments are methods for proliferating epithelial cells ex vivo, comprising expanding the number of cells in an originating epithelial cell population derived from differentiated tissue under serum-free and feeder-cell free conditions, thereby generating an expanded epithelial cell population, where the expansion culture conditions comprise an agent that activates telomerase reverse transcriptase in the population and/or inhibits transforming growth factor beta (TGF-beta) signaling in the population; and the originating epithelial cell population comprises quiescent and/or formerly quiescent epithelial cells. In certain embodiments, the expansion culture conditions comprise a second agent that modulates cytoskeletal structure in the population and the control culture conditions do not include the second agent.

Also provided herein in certain embodiments are methods for proliferating epithelial cells ex vivo, comprising expanding the number of cells in an originating epithelial cell population derived from differentiated tissue, embryonic stem (ES) cells, or induced pluripotent stem cells (iPSCs) under feeder-cell free expansion culture conditions, thereby generating an expanded epithelial cell population, where the expansion culture conditions comprise an agent that activates telomerase reverse transcriptase in the population and/or inhibits transforming growth factor beta (TGF-beta) signaling in the population; the originating epithelial cell population is capable of 25 population doublings or more when cultured under the expansion culture conditions; and the originating epithelial cell population is capable of no more than 20 population doublings when cultured under control culture conditions that do not include the agent.

Also provided herein in certain embodiments are methods for proliferating epithelial cells ex vivo, comprising expanding the number of cells in an originating epithelial cell population under serum-free and feeder-cell free conditions, thereby generating an expanded epithelial cell population, where the expansion culture conditions comprise an agent that activates telomerase reverse transcriptase in the population and/or inhibits transforming growth factor beta (TGF-beta) signaling in the population; and the originating epithelial cell population comprises differentiated epithelial cells.

Also provided herein in certain embodiments are methods for proliferating epithelial cells ex vivo, comprising expanding the number of cells in an originating epithelial cell population under serum-free and feeder-cell free conditions, thereby generating an expanded epithelial cell population, where the expansion culture conditions comprise an agent that activates telomerase reverse

transcriptase in the population and/or inhibits transforming growth factor beta (TGF-beta) signaling in the population; and the originating epithelial cell population comprises quiescent and/or formerly quiescent epithelial cells epithelial cells.

Also provided herein in certain embodiments are methods for proliferating epithelial cells ex vivo, comprising expanding the number of cells in an originating epithelial cell population serum-free and feeder-cell free conditions, thereby generating an expanded epithelial cell population, where the expansion culture conditions comprise an agent that activates telomerase reverse transcriptase in the population and/or inhibits transforming growth factor beta (TGF-beta) signaling in the population; and the originating epithelial cell population comprises lineage-committed epithelial cells.

Certain embodiments are described further in the following description, examples, claims and drawings.

Brief Description of the Drawings

The drawings illustrate certain embodiments of the technology and are not limiting. For clarity and ease of illustration, the drawings are not made to scale and, in some instances, various aspects may be shown exaggerated or enlarged to facilitate an understanding of particular embodiments.

Fig. 1 shows growth of prostate epithelial cells in regular culture medium (i.e., control culture conditions). KSFM, Keratinocyte-SFM (Gibco/Thermo Fisher). PrEGM, Prostate Epithelial Cell Growth Medium (Lonza). PD, population doublings.

Fig. 2 shows growth of bronchial epithelial cells in regular culture medium (i.e., control culture conditions). KSFM, Keratinocyte-SFM (Gibco/Thermo Fisher). PrEGM, Prostate Epithelial Cell Growth Medium (Lonza). PD, population doublings.

Fig. 3A and Fig. 3B show hTERT expression in epithelial cells. HBEC, human bronchial epithelial cells. hTERT, human telomerase reverse transcriptase gene. KSFM, Keratinocyte-SFM (Gibco/Thermo Fisher). LNCaP, human prostate cancer cell line LNCaP Clone FGC (Sigma-Aldrich). PrEC, prostate epithelial cells. PrEGM, Prostate Epithelial Cell Growth Medium (Lonza). n.d., non-detected. p, passage.

What is claimed is:

1. A method for proliferating epithelial cells ex vivo, comprising:

expanding the number of cells in an originating epithelial cell population comprising differentiated epithelial cells under serum-free and feeder-cell free expansion culture conditions, thereby generating an expanded epithelial cell population, wherein the expansion culture conditions comprise one or more agents that inhibit transforming growth factor beta (TGF-beta) signaling in the population, and

activating telomerase reverse transcriptase in the epithelial cell population.

2. The method of claim 1, wherein the one or more agents that inhibit transforming growth factor beta (TGF-beta) signaling in the population comprise one or more transforming growth factor beta (TGF-beta) receptor inhibitors.

3. The method of claim 1 or 2, the one or more agents that inhibit transforming growth factor beta (TGF-beta) signaling in the population comprise one or more ALK5 inhibitors.

4. The method of claim 3, wherein the one or more ALK5 inhibitors are selected from A83-01, GW788388, RepSox, and SB 431542.

5. The method of any one of claims 1 to 4, wherein the expansion culture conditions comprise a second agent that modulates cytoskeletal structure in the population.

6. The method of claim 5, wherein the second agent comprises an inhibitor selected from a Rho-associated protein kinase inhibitor, a p21-activated kinase (PAK) inhibitor, and a myosin II inhibitor.

7. The method of claim 6, wherein the Rho-associated protein kinase inhibitor is selected from Y-27632, SR 3677, thiazovivin, HA1100 hydrochloride, HA1077 and GSK-429286.

8. The method of claim 6, wherein the PAK inhibitor is IPA3.

9. The method of claim 6, wherein the myosin II inhibitor is blebbistatin.

10. The method of any one of claims 1 to 9, wherein the expansion culture conditions further comprise a beta-adrenergic receptor agonist.

11. The method of claim 10, wherein the beta-adrenergic receptor agonist is isoproterenol.

12. The method of any one of claims 1 to 11, wherein the expansion culture conditions comprise calcium at a concentration below 200 μM.

13. The method of any one of claims 1 to 11, wherein the expansion culture conditions comprise calcium at a concentration below 100 μM.

14. The method of any one of claims 1 to 13, wherein the expansion culture conditions comprise one or more mitogenic growth factors.

15. The method of claim 14, wherein the one or more mitogenic growth factors comprise EGF, FGF, or EGF and FGF.

16. The method of any one of claims 5 to 15, wherein the originating epithelial cell population is capable of 25 population doublings or more when cultured under the expansion culture conditions; and the originating epithelial cell population is capable of no more than 20 population doublings when cultured under control culture conditions that do not include the one or more agents that inhibit transforming growth factor beta (TGF-beta) signaling in the population.

17. The method of claim 16, wherein the originating epithelial cell population is capable of 50 population doublings or more when cultured under the expansion culture conditions.

18. The method of claim 16, wherein the originating epithelial cell population is capable of 80 population doublings or more when cultured under the expansion culture conditions.

19. The method of claim 16, wherein the originating epithelial cell population is capable of 100 population doublings or more when cultured under the expansion culture conditions.

20. The method of any one of claims 1 to 19, wherein the originating epithelial cell population is capable of expanding 5-fold or more over 5 days when cultured under the expansion culture

conditions; and the originating epithelial cell population is capable of expanding no more than 2-fold over 5 days when cultured under control culture conditions that do not include the one or more agents that inhibit transforming growth factor beta (TGF-beta) signaling in the population.

21. The method of any one of claims 1 to 19, wherein the originating epithelial cell population is capable of expanding 10-fold or more over 5 days when cultured under the expansion culture conditions; and the originating epithelial cell population is capable of expanding no more than 2-fold over 5 days when cultured under control culture conditions that do not include the one or more agents that inhibit transforming growth factor beta (TGF-beta) signaling in the population.

22. The method of any one of claims 1 to 21, wherein the expansion culture conditions do not comprise one or more of extracellular matrix comprising heterogeneous components, a Wnt agonist or a beta-catenin agonist.

23. The method of any one of claims 1 to 22, wherein the originating epithelial cell population is derived from differentiated tissue.

24. The method of any one of claims 1 to 22, wherein the originating epithelial cell population is derived from embryonic stem (ES) cells.

25. The method of any one of claims 1 to 22, wherein the originating epithelial cell population is derived from induced pluripotent stem cells (iPSCs).

26. The method of any one of claims 1 to 22, wherein the originating epithelial cell population does not comprise pluripotent cells.

27. A method for genetically modifying cells, comprising contacting cells with one or more agents that introduce a genetic modification to the cells, wherein the cells have been expanded by a method comprising:

expanding the number of cells in an originating epithelial cell population comprising differentiated epithelial cells under serum-free and feeder-cell free expansion culture conditions, thereby generating an expanded epithelial cell population, wherein the expansion culture conditions comprise one or more agents that inhibit transforming growth factor beta (TGF-beta) signaling in the population, and

activating telomerase reverse transcriptase in the epithelial cell population.

28. The method of claim 27, the one or more agents that inhibit transforming growth factor beta (TGF-beta) signaling in the population comprise one or more ALK5 inhibitors.

29. The method of claim 28, wherein the one or more ALK5 inhibitors are selected from A83-01, GW788388, RepSox, and SB 431542.

30. The method of claim 27, 28 or 29, wherein the expansion culture conditions comprise a second agent that modulates cytoskeletal structure in the population.

31. The method of claim 30, wherein the second agent comprises an inhibitor selected from a Rho-associated protein kinase inhibitor, a p21-activated kinase (PAK) inhibitor, and a myosin II inhibitor.

32. The method of claim 31, wherein the Rho-associated protein kinase inhibitor is selected from Y-27632, SR 3677, thiazovivin, HA1100 hydrochloride, HA1077 and GSK-429286.

33. The method of claim 31, wherein the PAK inhibitor is IPA3.

34. The method of claim 31, wherein the myosin II inhibitor is blebbistatin.

35. The method of any one of claims 27 to 34, wherein the expansion culture conditions comprise calcium at a concentration below 100 μM.