Claims

1. A method of on-column stimulation of T cells, the method comprising:

incubating, in a chromatography column, a sample comprising a plurality of T cells with one or more stimulatory agents to deliver a stimulatory signal in one or more T cells of the plurality of T cells, wherein:

the chromatography column comprises a housing assembly for column chromatography, the housing assembly comprising:

(i) an inlet housing member and an outlet housing member, wherein at least the inlet housing member and the outlet housing member form an internal cavity configured to house a stationary phase for column chromatography, wherein;

the internal cavity comprises the stationary phase for column chromatography; and

the stationary phase comprises an affinity chromatography matrix;

(ii) a temperature control member configured to provide heat to the stationary phase in the internal cavity; and

(iii) a connector configured to operably connect the internal cavity to a gas source, thereby permitting or effecting intake of gas into the internal cavity;

wherein the plurality of T cells are immobilized on the stationary phase; and

after the initiation of the incubation, collecting the one or more T cells from the stationary phase, thereby generating an output composition comprising stimulated T cells.

2. A method of on-column stimulation of T cells, the method comprising:

(a) adding a sample comprising a plurality of T cells to an internal cavity of a chromatography column, wherein:

the chromatography column comprises a housing assembly for column chromatography, the housing assembly comprising:

(i) an inlet housing member and an outlet housing member, wherein at least the inlet housing member and the outlet housing member form the internal cavity comprising a stationary phase for column chromatography;

(ii) a temperature control member configured to provide heat to the stationary phase in the internal cavity; and

(iii) a connector configured to operably connect the internal cavity to a gas source, thereby permitting or effecting intake of gas into the internal cavity;

wherein the stationary phase comprises an affinity chromatography matrix comprising a selection agent that binds to a selection marker on the surface of one or more of the plurality of T cells, thereby immobilizing the one or more of the plurality of T cells on the stationary phase;

(b) adding, to the stationary phase in the chromatography column, a stimulatory reagent comprising one or more stimulatory agent capable of delivering a stimulatory signal in one or more of the plurality of T cells, thereby initiating incubation of the stimulatory reagent with the one or more T cells; and

(c) after the initiation of the incubation, collecting the one or more T cells from the stationary phase, thereby generating a composition comprising stimulated T cells.

3. A method of on-column stimulation of T cells, comprising:

(a) combining (i) a sample comprising a plurality of T cells and (ii) a stationary phase for column chromatography, the stationary phase comprises an affinity chromatography matrix and a selection agent capable of specifically binding to a selection marker expressed on the surface of one or more of the plurality of T cells, wherein specific binding of the selection agent to a selection marker effects the immobilization of the plurality of T cells on the stationary phase;

(b) adding, to the stationary phase, a stimulatory reagent comprising one or more stimulatory agent capable of delivering a stimulatory signal in T cells, thereby initiating incubation of the stimulatory reagent with the one or more T cells,

wherein the combining step and/or the adding step is performed inside or outside the internal cavity of a chromatography column, wherein:

the chromatography column comprises a housing assembly for column chromatography, the housing assembly comprising:

(i) an inlet housing member and an outlet housing member, wherein at least the inlet housing member and the outlet housing member form the internal cavity comprising the stationary phase for column chromatography;

(ii) a temperature control member configured to provide heat to the stationary phase in the internal cavity; and

(iii) a connector configured to operably connect the internal cavity to a gas source, thereby permitting or effecting intake of gas into the internal cavity; and

(c) after the initiation of the incubation, collecting the one or more T cells from the stationary phase, thereby generating a composition comprising stimulated T cells.

4. The method of claim 1, wherein the stationary phase comprises an affinity chromatography matrix comprising a selection agent, wherein the selection agent of the stationary phase specifically binds to a selection marker on the surface of the plurality of T cells, wherein specific binding of the selection agent to the selection marker effects the immobilization of the plurality of T cells on the stationary phase.

5. The method of any of claims 1-4, wherein at least one of the one or more stimulatory agents is capable of delivering a stimulatory signal through an ITAM-containing molecule in a T cell.

6. The method of claim 5, wherein the at least one of the one or more stimulatory agents is capable of delivering a stimulatory signal through a TCR/CD3 complex.

7. The method of claim 5 or claim 6, wherein the at least one of the one or more stimulatory agents is a first stimulatory agent and the one or more stimulatory agents further comprise one or more of a second stimulatory agent capable of specifically binding to a costimulatory molecule on the T cell.

8. The method of claim 7, wherein the costimulatory molecule is CD28, CD90 (Thy-1), CD95 (Apo-/Fas), CD137 (4-1BB), CD154 (CD40L), ICOS, LAT, CD27, OX40, or HVEM.

9. The method of claim 7 or claim 8, wherein the first stimulatory agent specifically binds CD3 and the second stimulatory agent specifically binds CD28.

10. The method of claim 9, wherein the first stimulatory agent comprises an antibody, antibody fragment, or proteinaceous binding molecule that binds to CD3 and the second stimulatory agent comprises an antibody, antibody fragment, or proteinaceous binding molecule that binds to CD28.

11. The method of claim 10, wherein the first stimulatory agent is an anti-CD3 Fab and the second stimulatory agent is an anti-CD28 Fab.

12. The method of any one of claims 7-11, wherein the first stimulatory agent and the second stimulatory agent, independently, further comprise a streptavidin-binding peptide.

13. The method of any one of claims 1-12, wherein during at least a portion of the incubation, the temperature control member regulates the temperature of the stationary phase to a target temperature between 30°C and 39°C.

14. The method of claim 13, wherein the target temperature is at 37°C.

15. The method of any one of claims 1-14, wherein during at least a portion of the incubation, the connector allows intake of gas into the internal cavity.

16. The method of claim 15, wherein the gas is sterile and is or comprises air.

17. The method of any of claims 1-16, wherein the housing assembly further comprising a side wall member, wherein the inlet housing member, the outlet housing member, and the side wall member form the internal cavity.

18. The method any of claims 1-17, wherein:

the inlet housing member comprises one or more inlets operably connected to the internal cavity to permit intake of an input composition into the internal cavity; and/or

the outlet housing member comprises one or more outlets operably connected to the internal cavity to permit or effect discharge of an output composition from the internal cavity.

19. The method of any one of claims 1-18, wherein the temperature control member comprises a heating element or a plurality of heating elements.

20. The method of claim 19, wherein the heating element and/or at least one of the plurality of heating elements is an electric heating element.

21. The method of claim 20, wherein the heating element is a metal plate, a metal rod, a metal wire, or a combination thereof.

22. The method of claim 20 or claim 21, wherein the heating element and/or at least one of the plurality of heating elements is an electromagnetic induction heating element comprising an induction heating coil surrounding a magnetizable core configured to provide heat to the stationary phase in the internal cavity.

23. The method of any one of claims 19-22, wherein the heating element and/or at least one of the plurality of heating elements is a non-electric heating element comprising a heating channel comprising an inlet and an outlet for a heated fluid.

24. The method of claim 23, wherein the non-electric heating element comprises a heating coil.

25. The method of claim 23 or claim 24, wherein the heated fluid is heated water.

26. The method of any one of claims 19-25, wherein the heating element and/or at least one of the plurality of heating elements is disposed outside the internal cavity.

27. The method of any one of claims 19-26, wherein the heating element and/or at least one of the plurality of heating elements surrounds at least a portion of the inlet housing member, at least a portion of the outlet housing member, and/or at least a portion of the side wall member.

28. The method of any one of claims 19-27, comprising a plurality of heating elements, wherein the plurality of heating elements are uniformly distributed around the circumference of the side wall member.

29. The method of any one of claims 19-28, wherein the housing assembly further comprises a jacket member comprising the heating element or at least one of the plurality of heating elements, wherein the jacket member is configured to surround at least a portion of the inlet housing member, at least a portion of the outlet housing member, and/or at least a portion of the side wall member.

30. The method of claim 29, wherein the jacket member is releasably connected together to surround the at least a portion of the inlet housing member, the at least a portion of the outlet housing member, and/or the at least a portion of the side wall member.

31. The method of claim 29 or claim 30, wherein the jacket member is configured to entirely surround the side wall member.

32. The method of any one of claims 1-31, wherein the stationary phase comprises a non-magnetic material, a non-ferromagnetic material, or non-paramagnetic material.

33. The method of any of claims 4-32, wherein the selection agent comprises an antibody, antibody fragment, or proteinaceous binding molecule that specifically binds to the selection marker.

34. The method of any of claims 4-33, wherein the selection agent comprises or is an anti-CD3 Fab, an anti-CD8 Fab, an anti-CD4 Fab, or an anti-CD27 Fab.

35. The method of any of claims 4-34, wherein the selection agent is bound indirectly to the stationary phase through a selection reagent to which the selection agent reversibly binds.

36. The method of any of claims 4-35, wherein the selection reagent comprises or is a mutein of streptavidin that reversibly binds a streptavidin-binding peptide.