Claims

1. A method of expanding T lymphocytes in a microfluidic device having a flow path and a sequestration pen fluidically connected to the flow path, wherein the flow path comprises a microfluidic channel, wherein the sequestration pen comprises an isolation region and a connection region fluidically connecting the isolation region to the microfluidic channel, and wherein the isolation region of the sequestration pen is an unswept region of the microfluidic device, the method comprising:

introducing one or more T lymphocytes into the sequestration pen in the microfluidic device;

contacting the one or more T lymphocytes with an activating agent; wherein the activating agent comprises at least one selected from the group comprising: i) CD3 and CD28 agonists, ii) anti-CD3 agonist antibody, iii) anti-CD28 agonist antibody, iv) anti-CD3 agonist antibody conjugated to a solid support, v) anti-CD28 agonist antibody conjugated to a solid support, vi) anti-CD3 agonist antibody conjugated to a solid support and a solution of soluble anti-CD28 agonist antibody, vii) a dendritic cell that has been contacted with an antigen prior to contacting the one or more T lymphocytes, viii) a peptide antigen complexed with an MHC molecule, and ix) a peptide-MHC complex conjugated to a solid support;

perfusing culture medium through the flow path of the microfluidic device for a period of time sufficient to allow the one or more T lymphocytes introduced into the sequestration pen to undergo expansion; and

exporting the expanded T lymphocytes from the microfluidic device,

wherein at least one inner surface of the sequestration pen comprises a coating material, wherein the coating material is covalently bound to the at least one inner surface of the sequestration pen, and wherein the coating material comprises a polymer comprising alkylene ether moieties, saccharide moieties, amino acid moieties, or a combination thereof.

2. The method of claim 1, wherein the sequestration pen has a volume of $5x10^5$ to $5x10^6$ cubic microns.

3. The method of claim 1, wherein the coating material comprises-one or more proteins.

4. The method of claim 1, wherein the coating material comprises dextran or poly-ethylene glycol moieties.

5. The method of claim 1, wherein the coating material comprises covalently linked molecules, wherein each of the covalently linked molecules includes a linking group, wherein the linking group is covalently bonded to the at least one inner surface of the sequestration pen.

6. The method of claim 5, wherein the linking group is a siloxy linking group.

7. The method of claim 1, wherein the coating material comprises molecules having a linking group and a cationic moiety and/or an anionic moiety, wherein the linking group is covalently bonded to the at least one inner surface.

8. The method of any one of claims 1-7, wherein the activating agent is covalently linked or stably bound to the coating material.

9. The method of claim 8, wherein the activating agent is stably bound to the coating material via a biotin-streptavidi linkage.

10. The method of any one of claims 1-9, wherein the one or more T lymphocytes are isolated from a peripheral blood sample taken from a subject or from a solid tumor sample of a subject.

11. The method of claim 10, wherein the solid tumor is a breast cancer, a cancer originating in the urinary tract, ureter, bladder, or urethra, renal cell carcinoma, testicular cancer, prostate cancer, or a cancer of the seminal vesicles, seminal ducts, or penis, ovarian cancer, uterine cancer, cervical cancer, vaginal cancer, or a cancer of the fallopian tubes, a cancer of the nervous system, neuroblastoma, retinoblastoma, intestinal cancer, colorectal cancer, lung cancer, or melanoma.

12. The method of claim 10, wherein the solid tumor is a medullary breast cancer, a mesothelioma, or a melanoma.

13. The method of claim 10, wherein the one or more T lymphocytes are from a population of T lymphocytes isolated from the peripheral blood sample or the solid tumor sample.

14. The method of claim 13, wherein the population is enriched for $CD3^+$ T lymphocytes.

15. The method of claim 13, wherein the population is enriched for $CD3^+CD4^+$ or $CD3^+CD8^+$ T lymphocytes.

16. The method of any one of claims 13-15, wherein the population is enriched for $CD45RA^+CD45RO^-$ T lymphocytes.

17. The method of any one of claims 13-15, wherein the population is enriched for $CD45RA^-$ $CD45RO^+$ T lymphocytes.

18. The method of any one of claims 13-17, wherein the population is enriched for $CCR7^+$ T lymphocytes or $CD62L^+$ T lymphocytes.

19. The method of any one of claims 13-18, wherein the population is depleted of $CD69^+$ T-lymphocytes or PD-1^+ and/or PD-$L1^+$ T-lymphocytes.

20. The method of any one of claims 1-19, wherein introducing the one or more T lymphocytes into the sequestration pen comprises flowing a fluid containing the one or more T lymphocytes into the microfluidic channel of the microfluidic device, selecting at least one T lymphocyte of the one or more T lymphocytes located in the microfluidic channel, and moving the at least one selected T lymphocyte into the sequestration pen.

21. The method of claim 20, wherein the at least one selected T lymphocyte is selected, at least in part, because its cell surface comprises the marker $CD3^+$, $CD4^+$, $CD8^+$, or any combination thereof.

22. The method of claim 21, wherein (i) the at least one selected T lymphocyte is selected, at least in part, because its cell surface comprises the markers $CD3^+$ and $CD4^+$ or (ii) the at least one selected T lymphocyte is selected, at least in part, because its cell surface comprises the markers $CD3^+$ and $CD8^+$.

23. The method of any one of claims 20-22, wherein the at least one selected T lymphocyte is selected, at least in part, because its cell surface comprises the marker(s) $CD45RA^+$, $CD45RO^+$, $CCR7^+$, $CD62L^+$, or any combination thereof.

24. The method of any one of claims 20-23, wherein the at least one selected T lymphocyte is selected, at least in part, because its cell surface is not $CD45RO^+$.

25. The method of claim 24, wherein introducing one or more T lymphocytes into the sequestration pen comprises labeling $CD45RO^+$ T lymphocytes and introducing one or more T lymphocytes not associated with a label indicative of CD45RO into the sequestration pen.

26. The method of any one of claims 20-23, wherein the at least one selected T lymphocyte is selected, at least in part, because its cell surface is not $CD45RA^+$.

27. The method of claim 26, wherein introducing one or more T lymphocytes into the sequestration pen comprises labeling $CD45RA^+$ T lymphocytes and introducing one or more T lymphocytes not associated with a label indicative of CD45RA into the sequestration pen.

28. The method of any one of claims 20-27, wherein the at least one selected T lymphocyte is selected, at least in part, because its cell surface is not $CD69^+$, not $PD\text{-}1^+$, and/or not $PD\text{-}L1^+$.

29. The method of any one of claims 20-28, wherein the at least one selected T lymphocyte is selected, at least in part, because it is labeled with an antigen associated with a fluorescent label.

30. The method of claim 29, wherein the antigen comprises a peptide which is complexed with a MHC protein.

31. The method of any one of claims 1-30, wherein the one or more T lymphocytes are contacted with the activating agent prior to being introduced into the sequestration pen.

32. The method of claim 31, wherein the one or more T lymphocytes are incubated with the activating agent for a period of at least one hour prior to being introduced into the microfluidic device or for a period of at least five hours prior to being introduced into the microfluidic device.

33. The method of claim 31 or 32, wherein introducing the one or more T lymphocytes into the sequestration pen further comprises introducing the activating agent into the sequestration pen.

34. The method of any one of claims 1-30, wherein the one or more T lymphocytes are contacted with the activating agent after being introduced into the sequestration pen.

35. The method of any one of claims 1-34, wherein the activating agent comprises anti-CD3 and/or anti-CD28 agonist antibodies.

36. The method of claim 35, wherein the activating agent comprises an anti-CD3 agonist antibody which is conjugated to a solid support, an anti-CD28 agonist antibody which is conjugated to a solid support, or soluble anti-CD28 agonist antibodies.

37. The method of any one of claims 1-34, wherein the activating agent comprises a dendritic cell that has been contacted with an antigen prior to contacting the one or more T lymphocytes, wherein the antigen is a tumor antigen identified through genomic analysis of tumor cells.

38. The method of claim 37, wherein the analyzed tumor cells are autologous with the one or more T lymphocytes.

39. The method of any one of claims 1-38, wherein the culture medium is perfused through the flow path of the microfluidic device for a period of at least 24 hours.

40. The method of any one of claims 1-32, wherein the culture medium comprises IL7, IL15, IL21, or any combination thereof.

41. The method of any one of claims 1-40, wherein 5 or fewer T lymphocyte(s) are introduced into the sequestration pen in the microfluidic device.

42. The method of any one of claims 1-41, wherein the expansion comprises at least three rounds of mitotic cell division, and/or wherein the method comprises exporting the expanded T lymphocytes from the microfluidic device.

43. The method of any one of claims 1-42, wherein the microfluidic device comprises a plurality of sequestration pens, and wherein one or more T lymphocytes is introduced into each sequestration pen of the plurality and contacted with the activating agent.

44. The method of any one of claims 1 to 43, wherein the connection region comprises a proximal opening into the microfluidic channel having a width W_{con} ranging from 20 microns to 100 microns and a distal opening into the isolation region.

45. The method of claim 44, wherein a length L_{con} of the connection region from the proximal opening to the distal opening is as least 1.0 times a width W_{con} of the proximal opening of the connection region.

46. The method of claim 45, wherein the length L_{con} of the connection region from the proximal opening to the distal opening is at least 2.0 times the width W_{con} of the proximal opening of the connection region.

47. The method of claim 45 or 46, wherein a height of the microfluidic channel at the proximal opening of the connection region is between 20 microns and 100 microns.

48. The method of any one of claims 1 to 47, wherein the microfluidic device comprises an enclosure enclosing a microfluidic circuit comprising the flow region and the sequestration pen, wherein the enclosure comprises a base, a microfluidic circuit material disposed on the base, and a cover which collectively define the microfluidic circuit.

49. The method of claim 48, wherein the cover and the base are part of a dielectrophoresis (DEP) mechanism for selectively inducing DEP forces on a micro-object, and wherein introducing the one or more T lymphocytes into the sequestration pen further comprises using dielectrophoresis (DEP) to select at least one T lymphocyte located in the microfluidic channel and move it into the sequestration pen.

50. A T lymphocyte produced according to the method of any one of claims 1 to 42.