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, 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; and

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.

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

3. The method of claim 1, wherein at least one inner surface of the sequestration pen comprises a coating material that comprises covalently linked molecules.

4. The method of claim 3, wherein the coating material comprises a polymer comprising alkylene ether moieties, saccharide moieties, amino acid moieties, or a combination thereof, .

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

6. The method of claim 3, wherein each of the covalently linked molecules includes a linking group and an alkyl moiety, wherein the linking group is covalently bonded to the at least one inner surface of the sequestration pen.

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

8. The method of claim 3, 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.

9. The method of claim 8, wherein the coating material comprises molecules having a linking group and a zwitterionic moiety.

10. The method of claim 3, wherein the activating agent is covalently linked or stably bound to the coating material.

11. The method of claim 10, wherein the activating agent is stably bound to the coating material via a biotin-streptavidin linkage.

12. The method of claim 1, 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.

13. The method of claim 12, 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.

14. The method of claim 13, wherein the solid tumor is a medullary breast cancer, a mesothelioma, or a melanoma.

15. The method of claim 13, 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.

16. The method of claim 15, wherein the population is enriched for $CD3^+$ T lymphocytes.

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

18. The method of claim 15, wherein the population is enriched for $CD45RA^+CD45RO^-$ T lymphocytes.

19. The method of claim 15, wherein the population is enriched for $CD45RA^-CD45RO^+$ T lymphocytes.

20. The method of claim 15, wherein the population is enriched for $CCR7^+$ T lymphocytes or $CD62L^+$ T lymphocytes.

21. The method of claim 15, wherein the population is depleted of $CD69^+$ T-lymphocytes or $PD\text{-}1^+$ and/or $PD\text{-}L1^+$ T-lymphocytes.

22. The method of claim 1, wherein introducing the one or more T lymphocytes into the sequestration pen comprises flowing a fluid containing the one or more T lymphocytes into a microfluidic channel of the microfluidic device, wherein the microfluidic channel is part of the flow path of the microfluidic device, and wherein the sequestration pen opens off of the microfluidic channel.

23. The method of claim 22, 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.

24. The method of claim 23, 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+.

25. The method of claim 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.

26. The method of claim 22, wherein the at least one selected T lymphocyte is selected, at least in part, because its cell surface is not $CD45RO^+$, optionally 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.

27. The method of claim 22, wherein the at least one selected T lymphocyte is selected, at least in part, because its cell surface is not $CD45RA^+$, optionally 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 claim 22, 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 claim 22, 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 claim 1, 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 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 claim 1, wherein the one or more T lymphocytes are contacted with the activating agent after being introduced into the sequestration pen.

35. The method of claim 1, wherein (i) 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 claim 36, wherein the tumor antigen is 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 claim 1, 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 claim 1, wherein the culture medium comprises IL7, IL15, IL21, or any combination thereof.

41. The method of claim 1, wherein 20 or fewer, 10 or fewer, 6-10, 5 or fewer, about 5, about 4, about 3, about 2, or 1 T lymphocyte(s) are introduced into the sequestration pen in the microfluidic device.

42. The method of claim 1, 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 claim 1, 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. A T lymphocyte produced according to the method of any one of claims 1 to 43.

45. The method of any one of claims 1 to 43, wherein the flow region comprises a microfluidic channel, and wherein each sequestration pen comprises an isolation region having a single opening, and a connection region fluidically connecting the isolation region to the microfluidic channel.

46. The method of claim 45, wherein the connection region comprises a proximal opening into the microfluidic channel having a width W_{con} ranging from about 20 microns to about 100 microns and a distal opening into the isolation region, and 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.

47. The method of claim 46, 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.

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

49. The method of any one of claims 1 to 39, 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.

50. The method of claim 49, 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.