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 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;

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 about $5x10^5$ to about $5x10^6$ cubic microns.

3. The method of claim 1, wherein the coating material comprises at least one of dextran, poly-ethylene glycol moieties, and one or more proteins.

4. The method of claim 1, wherein the coating material comprises molecules comprising 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.

5. The method of claim 4, wherein the linking group is a siloxy linking group.

6. The method of claim 4 or claim 5, wherein:

the alkyl moiety comprises a linear chain of carbons comprising at least 10 carbon atoms; and/or

the alkyl moiety is a fluoroalkyl moiety or perfluoroalkyl moiety.

7. The method of any one of claims 4 to 6, wherein the molecules of the coating material form a densely-packed monolayer structure covalently bound to the inner substrate surface.

8. The method of any one of claims 1 to 7, 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 inner substrate surface.

9. The method of claim 8, wherein the coating material comprises at least one of:

the cationic moiety of the coating material comprises a quaternary ammonium group;
the anionic moiety of the coating material comprises a phosphonic acid, carboxylic acid, or sulfonic acid; and
the coating material comprises molecules having a linking group and a zwitterionic moiety.

10. The method of any one of claims 1 to 9, wherein the activating agent is covalently linked to the coating material or is stably bound to the coating material via a biotin-streptavidin linkage

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

12. The method of claim 11, wherein the one or more T lymphocytes are from the population of T lymphocytes isolated from the solid tumor sample of the subject, wherein the solid tumor is a breast cancer, lung cancer, or melanoma.

13. The method of claim 11 or claim 12, wherein the method further comprises enriching the population for $CD3^+$ T lymphocytes, $CD3^+CD4^+$ T lymphocytes, or $CD3^+CD8^+$ T lymphocytes.

14. The method of any one of claims 11 to 13, wherein method further comprises at least one of:

enriching the population for $CD45RA^+CD45RO^-$ T lymphocytes or $CD45RA^-CD45RO^+$ T lymphocytes;
enriching the population for $CCR7^+$ T lymphocytes;
enriching the population for $CD62L^+$ T lymphocytes;
depleting the population of $CD69^+$ T-lymphocytes;
depleting the population of PD-1^+ and/or PD-$L1^+$ T-lymphocytes; and
depleting the population of one, two, three, or four of $CD45RO^+$ T lymphocytes, $CD45RA^+$ T lymphocytes, $CD69^+$ T lymphocytes, PD-1^+ T lymphocytes, and PD-$L1^+$ T lymphocytes, wherein depleting comprises contacting the T lymphocytes with antibodies to CD45RO, CD45RA, CD69, PD-1, and/or PD-L1 and removing T lymphocytes bound to the antibodies from the population.

15. The method of any one of claims 1 to 14, 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 and using dielectrophoresis (DEP) to select at least one T lymphocyte located in the microfluidic channel and move it into the sequestration pen.

16. The method of claim 15, 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.

17. The method of claim 16, wherein the at least one T lymphocyte is selected, at least in part, when its cell surface is:

$CD3^+$, $CD4^+$, $CD8^+$, or any combination thereof; and/or
$CD45RA^+$, $CD45RO^+$, $CCR7^+$, $CD62L^+$, or any combination thereof.

18. The method of claim 16 or claim 17, wherein the at least one selected T lymphocyte is selected, at least in part, because its cell surface is not CD45RO+ is not CD45RA+.

19. The method of any one of claims 16 to 18, comprising labeling $CCR7^+$ and $CD62L^+$ T lymphocytes and

moving the T lymphocytes associated with a label indicative of CCR7 and/or associated with a label indicative of CD62L into the sequestration pen; or
moving the T lymphocytes not associated with a label indicative of CCR7 and/or not associated with a label indicative of CD62L into the sequestration pen.

20. The method of any one of claims 16 to 19, wherein the at least one T lymphocyte is selected, at least in part, when at least one of:

its cell surface is at least one of not $CD69^+$, not PD-1^+, and not PD-$L1^+$; and
its cell surface is labeled with an antigen associated with a fluorescent label.

21. The method of any one of claims 1 to 20, wherein the one or more T lymphocytes are contacted with the activating agent prior to being introduced into the sequestration pen.

22. The method of any one of claims 1 to 20, wherein the one or more T lymphocytes are contacted with the activating agent after being introduced into the sequestration pen.

23. The method of any one of claims 1 to 22, wherein the activating agent comprises at least one of an anti-CD3 agonist antibody which is conjugated to a solid support, and an anti-CD28

agonist antibody, wherein the anti-CD28 agonist antibody is conjugated to a solid support or is a soluble anti-CD28 agonist antibody.

24. The method of any one of claims 1 to 23, wherein the culture medium comprises human serum and IL2, and/or wherein the culture medium comprises IL7, IL15, IL21, or any combination thereof.

25. The method of any one of claims 1 to 24, 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.

26. The method of any one of claims 1 to 25, wherein 5 or fewer T lymphocyte(s) are introduced into the sequestration pen in the microfluidic device.

27. The method of any one of claims 1 to 26, wherein the connection region of the sequestration pen 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 said isolation region, and wherein a length L_{con} of said 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.

28. The method of claim 27, wherein the length L_{con} of said connection region from the proximal opening to the distal opening is at least 1.5 or 2.0 times the width W_{con} of the proximal opening of the connection region.

29. A T lymphocyte produced according to the method of claim 12, 23 or 26.

30. A medicament for use in the treatment of cancer in a subject, the medicament comprising

T lymphocytes isolated from a tissue sample obtained from the subject and expanded in a microfluidic device according to the method of claim 12, 23 or 26.

31. The medicament for use of claim 30, wherein the subject is a mammal.

32. The medicament for use of claims 30 or claim 31, wherein the tissue sample is a sample of peripheral blood or is from a solid tumor.

33. Use of T lymphocytes isolated from a tissue sample obtained from the subject and expanded in a microfluidic device according to the method of claim 12, 23 or 26 in the preparation of a medicament for the treatment of cancer in a subject.

34. The use of claim 33, wherein the subject is a mammal.

35. The use of claims 33 or 34, wherein the tissue sample is a sample of peripheral blood or is from a solid tumor.