CLAIMS

What is claimed is:

1. A microfluidic device comprising:

a body defining a first microchannel and a first chamber, the first microchannel having an inlet and an outlet to an exterior of the body, and the first chamber being in fluidic communication with the first microchannel; and

a matrix filling the first chamber and comprising B lymphocytes, the B lymphocytes being arranged in clusters within the matrix, the clusters containing less than 10% T lymphocytes.

2. The microfluidic device of claim 1, further comprising a second chamber and a second matrix, the second matrix filling at least a portion of the second chamber.

3. The microfluidic device of claim 2, wherein said second matrix further comprises T lymphocytes.

4. The microfluidic device of claim 1, wherein the matrix is an extracellular matrix.

5. The microfluidic device of claim 4, wherein the extracellular matrix comprises a hydrogel.

6. The microfluidic device of claim 5, wherein the extracellular matrix comprises a fibrillar network of collagen, laminin, and heparin sulfate proteoglycan.

7. The microfluidic device of claim 6, wherein the extracellular matrix comprises MATRIGEL.

8. The microfluidic device of claim 4, wherein the matrix is formed of a 50:50 mix of MATRIGEL and culture medium.

9. The microfluidic device of claim 8, wherein the culture medium is RPMI medium.

10. The microfluidic device of claim 1, further comprising a reticular network within the matrix to facilitate lymphocyte scanning by one or more of the T lympohcytes or B lymphocytes.

11. The microfluidic device of claim 1, wherein the T lympohcytes and B lymphocytes are present within the matrix in an amount of approximately 100,000,000 per milliliter.

12. The microfluidic device of claim 1, wherein at least a portion of the first microchannel is lined with vascular endothelial cells to mimic a vascular vessel.

13. The microfluidic device of claim 1, wherein at least a portion of the first microchannel is lined with lymphatic endothelial cells.

14. The microfluidic device of claim 1, further comprising a membrane separating the first microchannel from the first chamber, wherein the membrane permits fluid and solids to pass between the first microchannel and the first chamber.

15. The microfluidic device of claim 14, wherein the membrane is porous.

16. The microfluidic device of claim 15, wherein the pores are 0.4 to 10 μm in diameter.

17. The microfluidic device of claim 16, wherein the pores are 0.4 to 1 μm in diameter.

18. The microfluidic device of claim 1, wherein the body forms a constriction at an interface of the first microchannel and the first chamber to reduce an interface area between the first microchannel and the first chamber as compared to a longitudinal cross-section of the first microchannel and the first chamber.

19. The microfluidic device of claim 1, wherein the clusters mimic germinal centers within human lymph nodes.

20. The microfluidic device of claim 1, further comprising one or more microdomains within the matrix formed of microbeads, nanoparticles, or a combination thereof.

21. The microfluidic device of claim 20, wherein the microdomains are configured to provide a controlled release of one or more bioactive components to promote three-dimensional organization of the T lymphocytes and the B lymphocytes.

22. The microfluidic device of claim 1, wherein the volume of the matrix is about 10 µL.

23. The microfluidic device of claim 1, where the first microchannel and the first chamber are about 200 mm long and about 1 mm wide.

24. The microfluidic device of claim 1, wherein the body further defines a second microchannel in fluidic communication with the first chamber and on an opposite side of the first chamber from the first microchannel.

25. The microfluidic device of claim 24, wherein the first microchannel is lined with blood vessel endothelial cells to mimic a blood vessel and the second microchannel is lined with lymphatic endothelial cells to mimic a lymphatic vessel.

26. The microfluidic device of claim 24, wherein the body further defines a second chamber in fluidic communication with and between the first chamber and the second microchannel.

27. The microfluidic device of claim 26, wherein the second chamber is filled with the matrix and predominantly T lymphocytes and the first chamber is filled predominantly with the B lymphocytes.

28. The microfluidic device of claim 27, wherein the first microchannel and the first chamber are circular.

29. A method comprising:

a) providing a microfluidic device comprising a body comprising a microchannel in fluidic communication with a chamber, the microchannel comprising a microchannel inlet and a microchannel outlet, the chamber comprising a matrix;

b) providing T lymphocytes and B lymphocytes within the matrix; and

c) culturing said T lymphocytes and B lymphocytes at a density such that the B lymphocytes form clusters within the matrix containing less than 10% T lymphocytes, wherein the density of the T and B lymphocytes within the matrix is greater than 500,000 cells per millilitre.

30. The method of claim 29, wherein the microchannel inlet, the microchannel and the microchannel outlet collectively define a fluid path, and wherein said fluid path is used to seed the matrix in step b) with the T lymphocytes and B lymphocytes.

31. The method of claim 29, wherein the matrix is an extracellular matrix.

32. The method of claim 31, wherein the extracellular matrix comprises a hydrogel.

33. The method of claim 30, wherein said T lymphocytes and B lymphocytes are seeded into the matrix by flowing peripheral blood mononuclear cells (PBMCs) through the fluid path.

34. The method of claim 30, wherein the T and B lymphocytes are seeded within the matrix in a ratio of about 40:60 to about 60:40 T lymphocytes to B lymphocytes.

35. The method of claim 29, wherein the density of the T and B lymphocytes within the matrix is 1 x 10^8 to about 2 x 10^8 cells per milliliter.

36. The method of claim 30, wherein the density of cells in the matrix is such that the B lymphocytes self-organize into clusters.

37. The method of claim 29, further comprising the step d) measuring lymphocyte proliferation and/or antigen specificity.

38. The method of claim 29, further comprising the step d) measuring lymphocyte cytokine and/or antibody secretion.

39. The method of claim 29, further comprising the step d) exposing said lymphocytes to an agent.

40. The method of claim 39, wherein said agent is selected from the group consisting of a cytokine, an antigen, and a drug.

41. The method of claim 39, wherein said agent is selected from the group consisting of tumor antigens, pathogens, and vaccines.

42. The method of claim 39, wherein said agent is selected from the group consisting of candidate drugs, known anti-cancer drugs, known checkpoint inhibitors, and candidate checkpoint inhibitors.

43. The method of claim 42, wherein the checkpoint inhibitor is an antibody.

44. The method of claim 43, wherein said antibody binds the PD-1 receptor on T cells.

45. The method of claim 39, wherein said matrix comprises said agent and said agent is released from the matrix.

46. The method of claim 45, wherein said matrix releases scaffolds comprising said agent.

47. The method of claim 46, wherein said released scaffolds comprise microbeads.

48. The method of claim 46, wherein said released scaffolds comprise nanoparticles.

49. The method of claim 48, wherein said nanoparticles release bioactive molecules.

50. The method of claim 29, further comprising the step of seeding the matrix with additional immune and/or stromal cells.

51. The method of claim 29, further comprising the step of flowing additional immune and/or stromal cells into the device.

52. The method of claim 50, wherein said additional immune and/or stromal cells form a reticular network within the matrix.

53. The method of claim 50, wherein the immune and/or stromal cells are selected from the group consisting of monocytes, macrophages and dendritic cells.

54. The method of claim 53, wherein the monocytes are CD14+.

55. The method of claim 53, wherein the monocytes are GM-CSF/IL-4 cultured monocytes.

56. The method of claim 52, the reticular network is an antigen presenting network.

57. The method of claim 53, wherein said macrophages and dendritic cells are monocyte-derived.

58. The method of claim 53, wherein said macrophages or dendritic cells carry or present an antigen or portion thereof.

59. The method of claim 58, wherein said antigen or portion thereof generates an immune response as measured by lymphocyte proliferation.

60. The method of claim 58, wherein said antigen or portion thereof generates an immune response as measured by lymphocyte secretion of cytokines or secretion of antibody.

61. The method of claim 29, wherein said B lymphocytes clusters contain less than 5% T lymphocytes.

62. The method of claim 29, wherein said B lymphocytes clusters contain less than 1% T lymphocytes.

63. The method of claim 29, wherein said culturing of step c) comprises perfusing said lymphocytes with culture media at a flow rate.

64. The method of claim 63, further comprising the step d) removing a portion of said culture media from set microchannel outlet or from said chamber.

65. The method of claim 64, further comprising the step e) testing said culture media for secreted molecules.

66. The method of claim 64, further comprising the step e) introducing said portion of said culture media into a different microfluidic device, said different microfluidic device comprising cells.

67. A method, comprising:

a) providing a microfluidic device comprising a body comprising a microchannel in fluidic communication with a chamber, the microchannel comprising a microchannel inlet and a microchannel outlet, the chamber comprising a matrix;

b) seeding T lymphocytes and B lymphocytes within the matrix in a ratio of 40:60 to 60:40 T lymphocytes to B lymphocytes;

c) culturing said seeded cells at a density such that the B lymphocytes form clusters within the matrix containing less than 10% T lymphocytes; and

d) exposing said lymphocytes to tumor cells.

68. The method of claim 67, wherein said exposing of step d) comprises introducing tumor cells into said device.

69. The method of claim 67, wherein said exposing of step d) comprises removing at least a portion of said lymphocytes from said device and exposing said removed cells to tumor cells outside said device.

70. The method of claim 67, wherein the microchannel inlet, the microchannel and the microchannel outlet collectively define a fluid path, and wherein said fluid path is used to seed the matrix in step b).

71. The method of claim 67, further comprising the step e) measuring lymphocyte proliferation.

72. The method of claim 67, further comprising the step e) measuring lymphocyte cytokine secretion and/or antibody secretion.

73. The method of claim 67, further comprising the step e) exposing said lymphocytes to an agent.

74. The method of claim 73, wherein said agent is selected from the group consisting of a cytokine, an antigen and a drug.

75. The method of claim 73, wherein said agent is selected from the group consisting of tumor antigens, pathogens, and vaccines.

76. The method of claim 73, wherein said agent is selected from the group consisting of candidate drugs, known anti-cancer drugs, known checkpoint inhibitors and candidate checkpoint inhibitors.

77. The method of claim 76, wherein the checkpoint inhibitor is an antibody.

78. The method of claim 77, wherein said antibody binds the PD-1 receptor on T cells.

79. The method of claim 78, wherein said antibody binds the PD-L1 ligand on the tumor cells.

80. The method of claim 73, wherein the method further comprises step of f) detecting the impact of the agent on the lymphocytes.

81. The method of claim 80, wherein said detecting comprises detecting tumor cell death by said lymphocytes.