Claims

1. A method of processing and storing biological cells, comprising:

introducing a flowable medium into a microfluidic device having a flow region, the flowable medium including biological cells;

sequestering one or more biological cells from the flowable medium in one or more isolation chambers of the microfluidic device, each of the one or more isolation chambers containing only a single opening that is fluidically connected to the flow region; and

freezing the microfluidic device including the one or more biological cells sequestered therein.

2. The method of claim 1, wherein a single biological cell is sequestered in each of a plurality of isolation chambers.

3. The method of claim 1 or claim 2, wherein each isolation chamber has a volume in a range of 1.5×10^5 cubic microns to 1.5×10^6 cubic microns.

4. The method of any one of claims 1-3, wherein each isolation chamber is configured to sequester 10 cells to 50 cells.

5. The method of any one of claims 1-4, further comprising, prior to freezing the micro-fluidic device, creating an inventory including at least an identity and isolation chamber location for each of the one or more sequestered biological cells, and storing the inventory in a digital memory associated with the microfluidic device.

6. The method of claim 5, the inventory further including information identifying one or more of (i) how the biological cells in the flowable medium were obtained, (ii) processing, if any, performed on the biological cells prior to or after their introduction into the microfluidic device, (iii) processing, if any, performed on the one or more sequestered biological cells after their sequester within the microfluidic device; and (iv) data obtained in the course of any such pre or post sequestration processing.

7. The method of claim 5 or claim 6, wherein the memory comprises a chip or other device coupled to, and frozen with, the microfluidic device.

8. The method of any one of claims 1-7, wherein freezing the microfluidic device comprises an initial controlled cooling of the microfluidic device to a temperature of 0°C, followed by a subsequent cooling of the microfluidic device to a subzero temperature.

9. The method of claim 8, wherein the initial controlled cooling comprises cooling the microfluidic device at a rate in a range of 0.1°C per minute to 2°C per minute.

10. The method of claim 8 or claim 9, wherein the subzero temperature is abut -20°C or less.

11. The method of claim 8 or claim 9, wherein the subzero temperature is -80°C or less.

12. The method of claim 8 or claim 9, wherein the subzero temp is -150°C or less.

13. The method of any one of claims 1-12, further comprising, prior to freezing the microfluidic device, introducing a cell preservation reagent into the microfluidic device.

14. The method of claim 13, wherein the cell preservation reagent comprises dimethyl sulfoxide (DMSO).

15. The method of claim 14, wherein the DMSO is introduced into the microfluidic device at a respective concentration and duration selected such that the one or more sequestered biological cells are surrounded by a solution containing 10% DMSO at the time of freezing the microfluidic device.

16. The method of claim 14, wherein the DMSO is introduced into the microfluidic device at a concentration of 15% to 25% by volume and allowed to diffuse
into the one or more isolation chambers containing sequestered biological cells.

17. The method of claim 14, wherein the DMSO is perfused through the microfluidic device for an amount of time sufficient to achieve a DMSO concentration of 10% in each of the one or more isolation chambers.

18. The method of any one of claims 1-17, further comprising thawing the microfluidic device.

19. The method of claim 18, further comprising, after thawing the microfluidic device, culturing one or more viable cells in the microfluidic device to thereby generate additional cells therein.

20. The method of claim 18 or claim 19, further comprising identifying which of the one or more sequestered cells and/or cells generated therefrom are viable after thawing the microfluidic device.

21. The method of any one of claims 18-20, further comprising, after thawing the microfluidic device, retrieving from the microfluidic device at least one sequestered cell and/or cells generated therefrom.

22. The method of any one of claims 18-21, wherein thawing the microfluidic device comprises one or both of (i) a controlled heating of the microfluidic device, and (ii) allowing the microfluidic device to self-heat to room temperature.

23. The method of any one of claims 1-22, the one or more sequestered biological cells comprising at least one or more starting cells sequestered in a first isolation chamber, the method further comprising:

prior to freezing the microfluidic device, culturing the one or more starting cells to generate a plurality of new cells in the first isolation chamber, the plurality of new cells being adequate in number so that at least one viable cell is located in the first isolation region after thawing the microfluidic device.

24. The method of claim 18 or claim 23, wherein culturing the cells comprises perfusing a flowable cell growth medium through the microfluidic device.

25. The method of claim 24, wherein the perfusion is continuous or intermittent

26. The method of claim 23, wherein the plurality of new cells comprises at least 8 cells for each starting cell.

27. The method of any one of claims 1-26, further comprising performing an assay of the one or more sequestered biological cells.

28. The method of claim 27, wherein the assay is used to detect a cell secretion or a cell surface marker.

29. The method of any one of claims 1-28, wherein the flow region and one or more isolation chambers are treated with a blocking solution to prevent or reduce cell adhesion.

30. The method of claim 29, wherein the blocking solution comprises serum, BSA, polymer, detergent, enzymes, or any combination thereof.

31. The method of any one of claims 1-30, wherein the microfluidic device comprises an inner substrate surface that comprises a coating material, wherein molecules of the coating material comprise a linking group that is covalently linked to the inner substrate surface.

32. The method of claim 31, wherein the molecules of the coating material comprise an alkyl moiety, and wherein the alkyl moiety comprises a linear chain of carbons comprising at least 10 carbon atoms.

33. The method of claim 32, wherein the alkyl moiety is a fluoroalkyl moiety.

34. The method of claim 32 or claim 33, wherein the linking group is a siloxy linking group.

35. The method of any one of claims 32-34, wherein the molecules of the coating material form a densely-packed monolayer structure covalently bound to the inner substrate surface.

36. The method of claim 31, wherein the molecules of the coating material comprise a cationic moiety and/or an anionic moiety.

37. The method of claim 36, wherein the cationic moiety comprises a quaternary ammonium group, and/or he anionic moiety comprises a phosphonic acid, carboxylic acid, or sulfonic acid.

38. The method of claim 36 or claim 37, wherein the molecules of the coating material comprise a zwitterionic moiety.

39. The method of claim 38, wherein the zwitterionic moiety is selected from carboxy-betaines, sulfobetaines, sulfamic acids, and amino acids.

40. The method of claim 31, wherein the coating material comprises a polymer comprising alkylene ether moieties, saccharide moieties, or amino acid moieties.

41. The method of claim 40, wherein the coating material comprises dextran.

42. The method of claim 40, wherein the coating material comprises polyethylene glycol.

43. The method of any one of claims 1 to 42, wherein each of the one or more isolation chambers comprises an isolation region having a single opening, and a connection region fluidically connecting the isolation region to the flow region.

44. The method of claim 43, wherein the flow region comprises a microfluidic channel, and wherein the connection region comprises a proximal opening into said microfluidic channel having a width W_{con} ranging from 20 microns to 100microns and a distal opening into said isolation region, and wherein a length L_{con} of said connection region from said proximal opening to said distal opening is as least 1.0 times a width W_{con} of said proximal opening of said connection region.

45. The method of claim 44, wherein the length L_{con} of said connection region from said proximal opening to said distal opening is at least 2.0 times the width W_{con} of said proximal opening of said connection region.

46. The method of any one of claims 43 to 45, wherein a height of said microfluidic channel at said proximal opening of said connection region is between 20 microns and 100 microns.

47. The method of any one of claims 1 to 46, wherein the microfluidic device comprises an enclosure enclosing a microfluidic circuit comprising the flow region and the one or more

isolation chamber, wherein the enclosure comprises a base, a microfluidic circuit material disposed on the base, and a cover which collectively define the microfluidic circuit.

48. The method of claim 47, wherein the cover and the base are part of a dielectrophoresis (DEP) mechanism for selectively inducing DEP forces on a micro-object, and

wherein the DEP forces are used to sequester the one or more biological cells in the one or more isolation chambers.