

[0009] Thus, what is needed are methods, compositions and apparatuses for generating patient derived tissue models (e.g., tumor models and/or non-tumor tissue models) from resection or biopsies. In particular, it would be useful to provide methods and apparatuses that may enable a large number of patient-derived tissue models having predictable and clinically relevant properties from a single biopsy, such as an 18-gauge core biopsy, which could be completed within, e.g., 7-10 days after obtaining a biopsy. This would permit robust and reliable testing and minimize delays in guiding patient-specific therapies. Furthermore, it will also be useful to generate patient derived models that can expand quickly in a highly parallel manner, generating units with smaller and more uniform sizes, better controllability for cell number per unit, and better diffusibility (e.g., via increase surface to volume ratio), for high-throughput screen applications.

SUMMARY ~~OF THE DISCLOSURE~~

[0009a] In one aspect, the present disclosure refers to a method, the method comprising:
forming a plurality of droplets of an unpolymerized mixture from a dissociated tissue sample and a fluid matrix material; and
polymerizing the droplets to form a plurality of Patient-Derived Micro-Organospheres each having a diameter of between 50 and 500 μm with between 1 and 200 dissociated cells distributed therein.

[0009b] In another aspect, the present disclosure refers to a method, the method comprising:
combining, within a temperature-controlled system maintained at less than 25 degrees C, a freshly dissociated tissue sample and a fluid matrix material comprising a substrate basement membrane matrix to form an unpolymerized mixture, wherein the dissociated tissue sample comprises a single biopsy or resected patient sample;
forming a plurality of droplets of the unpolymerized mixture; and
polymerizing the droplets by increasing the temperature to greater than 30 degrees C to form more than a thousand Patient-Derived Micro-Organospheres each having a diameter of between 50 and 500 μm with between 1 and 200 dissociated cells distributed therein,
culturing the plurality of Patient-Derived Micro-Organospheres for between 3-10 days of forming the droplets to form structured clusters of cells replicating the structures of the tissue from which they were biopsied or resected.

[0010] Described herein are Patient-Derived Micro-Organospheres (PMOSs), apparatuses and methods of making PMOSs, and apparatuses and methods of using PMOSs. Also described herein are methods and systems for screening a patient using these Patient-Derived Micro-Organospheres, including personalized therapy methods.

[0011] In general, described herein are methods and apparatuses that form and grow Patient-Derived Micro-Organospheres containing cells originating from a patient, for example, extracted from a small patient biopsy, (e.g., for quick diagnostics to guide therapy), from resected patient tissue, including resected primary tumor or part of a dysfunctional organ (e.g., for high-throughput screening), and/or from already established PDMCs, including patient-derived xenografts (PDX) and organoids (e.g., to generate Micro-Organospheres for high-throughput screening).

[0012] These PMOSs may be formed from primary cells that are normal (e.g., normal organ tissue) or from tumor tissue. For example, in some variations, these methods and apparatuses may form PMOSs from cancerous tumor biopsy tissue, enabling tailored treatments that can be selected using the particular tumor tissue examined. Surprisingly, these methods and apparatuses permit the formation of hundreds, thousands or even tens of thousands (e.g., 500, 750, 1000, 2000, 5000, 10,000 or more) of PMOSs from a single tissue biopsy, within a few hours of the biopsy being removed from the patient. Dissociated primary cells from the patient biopsy may be combined with a fluid matrix material, such as a substrate basement membrane matrix (e.g., MATRIGEL), to form the micro-organosphere. The resulting plurality of Patient-Derived Micro-Organospheres may have a predefined range of sizes (such as diameters, e.g., between 10 μm and 700 μm and any sub-range therewithin), and initial number of primary cells (e.g., between 1

CLAIMS

What is claimed is:

1. A method, the method comprising:

~~combining a dissociated tissue sample and a fluid matrix material to form an unpolymerized mixture;~~

forming a plurality of droplets of ~~an~~the unpolymerized mixture from a dissociated tissue sample and a fluid matrix material; and

polymerizing the droplets to form a plurality of Patient-Derived Micro-

Organospheres each having a diameter of between 50 and 500 μm with between 1 and 200 dissociated cells distributed therein.

~~2. A method, the method comprising:~~

~~combining a dissociated tissue sample and a fluid matrix material to form an unpolymerized mixture;~~

~~forming a plurality of droplets from a continuous stream of the unpolymerized mixture wherein the droplets have less than a 25% variation in size; and~~

~~polymerizing the droplets by warming to form a plurality of Patient-Derived Micro-Organospheres each having between 1 and 200 dissociated cells distributed within each Patient-Derived Micro-Organosphere.~~

~~3. A method, the method comprising:~~

~~combining a dissociated tissue sample and a fluid matrix material to form an unpolymerized mixture;~~

~~forming a plurality of droplets having less than a 25% variation in a size of the droplets by converging a stream of the unpolymerized mixture with one or more streams of a fluid that is immiscible with the unpolymerized mixture;~~

~~polymerizing the droplets to form a plurality of Patient-Derived Micro-~~

~~Organospheres having a diameter of between 50 and 500 μm with between 1 and 200 dissociated cells distributed therein; and~~

~~separating the plurality of Patient-Derived Micro-Organospheres from the fluid that is immiscible.~~

4.2. The method of ~~any of claims 1-3~~, further comprising modifying the cells within the dissociated tissue sample prior to forming the droplets.

5.3. The method of claim 1, wherein forming the plurality of droplets comprises forming a plurality of droplets of the unpolymerized mixture of uniform size with less than 25% variation in size.

6. ~~The method of any of claims 1-5, further comprising combining Patient-Derived Micro-Organospheres from various sources into a receptacle prior to assaying.~~

7. ~~The method of any of claims 1-6, further comprising applying the Patient-Derived Micro-Organospheres into a receptacle comprising a plurality of chambers separated by an at least partially permeable membrane to permit circulation of supernatant material between the chambers.~~

8. ~~The method of any of claims 1-7, further comprising recovering one or more of the Patient-Derived Micro-Organospheres following the assay for further assaying or expansion.~~

9. ~~The method of any of claims 1-8, wherein assaying the plurality of Patient-Derived Micro-Organospheres comprises sampling one or more of a supernatant, an environment, and a microenvironment of the Patient-Derived Micro-Organospheres for secreted factors and other effects.~~

10.4. The method of ~~any of claims 1-9~~, wherein the tissue sample comprises cells that are not stem cells.

11.5. The method of ~~any of claims 1-10~~, wherein the tissue sample comprises a biopsy sample from a metastatic tumor.

12.6. The method of ~~any of claims 1-10~~, wherein the tissue sample comprises a clinical tumor sample, further wherein the clinical tumor sample comprises both cancer cells and stroma cells.

13.7. The method of ~~any of claims 1-10~~, wherein the tissue sample comprise tumor cells and one or more of: mesenchymal cells, endothelial cells, and immune cells.

14.8. The method of ~~any of claims 1-10~~, wherein combining the dissociated tissue sample and the fluid matrix material comprises disbursing the dissociated tissue sample within the fluid matrix material to a density of less than 5×10^6 cells/ml.

15. ~~The method of any of claims 1-14, wherein forming the droplet comprises forming the droplet from a continuous stream of the unpolymerized mixture.~~

16. ~~The method of claim 15, wherein forming the droplet comprises applying one or more convergent streams of a fluid that is immiscible with the unpolymerized mixture to the stream of unpolymerized mixture.~~

17. ~~The method of any of claims 1 or 2, wherein forming the droplet comprises forming the droplet in a fluid that is immiscible with the unpolymerized mixture.~~

18.9. The method of ~~any of claims 83 or 17~~, further comprising removing the immiscible fluid from the Patient-Derived Micro-Organosphere.

10. The method of claim 1, wherein the tissue sample is combined with the fluid matrix material within six hours of removing the tissue sample from the patient.

11. The method of claim 1, wherein forming the droplet comprises forming the droplet in a fluid that is immiscible with the unpolymerized mixture.

12. The method of claim 11, wherein the temperature of the immiscible fluid is maintained at greater than 30 degrees C.

13. The method of ~~any of claims 1-18~~, wherein combining the dissociated tissue sample and the fluid matrix material comprises combining the dissociated tissue sample with a MATRIGEL substrate basement membrane matrix.

~~20. The method of any of claims 1-19, wherein the tissue sample is combined with the fluid matrix material within six hours of removing the tissue sample from the patient.~~

~~21. The method of any of claims 1-20, further comprising dispensing the Patient-Derived Micro-Organospheres into a multi-well plate prior to assaying the Patient-Derived Micro-Organosphere.~~

~~22. The method of any of claims 1-21, wherein polymerizing comprises heating the droplet to greater than 35 degrees C.~~

14. The method of claim 1, further comprising screening the plurality of Patient-Derived Micro-Organospheres with a plurality of drug compositions within 14 days of acquiring the biopsy or resected patient sample.

15. The method of claim 1, further comprising screening the plurality of Patient-Derived Micro-Organospheres with hundreds of drug compositions within 14 days of acquiring the biopsy or resected patient sample.

16. The method of claim 1, wherein culturing comprises culturing the plurality of Patient-Derived Micro-Organospheres for between 2-14 days of forming the droplets to form clusters of cells replicating the structures of the tissue from which they were biopsied or resected.

17. The method of claim 1, wherein the dissociated tissue sample comprises epithelial cells, further wherein culturing comprises culturing the plurality of Patient-Derived Micro-Organospheres for between 2-14 days of forming the droplets to form cells replicating the structures of the tissue from which they were biopsied or resected.

18. The method of claim 11, further comprising monitoring the flow rate of the unpolymerized mixture and the immiscible fluid to maintain a continuous stream of unpolymerized mixture during the method.

19. The method of claim 1, further comprising pressurizing the unpolymerized material.

~~23. A method, the method comprising:~~

~~combining a dissociated tissue sample and a fluid matrix material to form an unpolymerized mixture;~~

~~forming a plurality of droplets of the unpolymerized mixture having less than a 25% variation in a size of the droplets;~~

~~polymerizing the droplets to form a plurality of Patient-Derived Micro-Organospheres having a diameter of between 50 and 700 μm with between 1 and 1000 dissociated cells distributed therein; and~~

~~assaying or cryopreserving the plurality of Patient-Derived Micro-Organospheres.~~

~~24. A method, the method comprising:~~

~~combining a dissociated tissue sample and a fluid matrix material to form an unpolymerized mixture;~~

~~forming a plurality of droplets of the unpolymerized mixture;~~

~~polymerizing the droplets to form a plurality of Patient-Derived Micro-~~

~~Organospheres each having a diameter of between 50 and 500 μm with between 1 and 200 dissociated cells distributed therein; and~~

~~cryopreserving or assaying the plurality of Patient-Derived Micro-Organospheres within 15 days, wherein the microoganoids are assayed to determine the effect of one or more agents on the cells within the Patient-Derived Micro-Organospheres.~~

25.20. A method, the method comprising:

5 ~~Combining~~combining, within a temperature-controlled system maintained at less than 25 degrees C, a freshly dissociated tissue sample and a fluid matrix material comprising a substrate basement membrane matrix to form an unpolymerized mixture, wherein the dissociated tissue sample comprises a single biopsy or resected patient sample;

10 forming a plurality of droplets ~~having less than a 25% variation in a size of the droplets by converging a stream~~ of the unpolymerized mixture ~~with one or more streams of a fluid that is immiscible with the unpolymerized mixture; and~~ polymerizing the droplets by increasing the temperature to greater than 30 degrees C ~~warming~~ to form more than a thousand Patient-Derived Micro-Organospheres each having a diameter of between 50 and 500 μm with between 1 and 200 dissociated cells distributed therein; ~~and~~

15 ~~assaying or cryopreserving~~culturing the plurality of the Patient-Derived Micro-Organospheres for between 3-10 days of forming the droplets to form structured clusters of cells replicating the structures of the tissue from which they were biopsied or resected.~~before six passages, whereby heterogeneity of the cells within the Patient-Derived Micro-Organospheres is maintained, further wherein assaying comprises assaying in order to determine the effect of one or more agents on the cells within the Patient-Derived Micro-Organosphere.~~

26. The method of claim 23 or 24, ~~wherein the plurality of Patient-Derived Micro-Organospheres are cryopreserved or assayed before six passages, whereby heterogeneity of the cells within the Patient-Derived Micro-Organospheres is maintained.~~

27. The method of any of claims 23-26, ~~further comprising modifying the cells within the dissociated tissue sample prior to forming the droplets.~~

28. The method of claims 24, ~~wherein forming the droplet comprises forming a plurality of droplets of the unpolymerized mixture of uniform size with less than 25% variation in size.~~

29. ~~The method of any of claims 23-28, further comprising combining Patient-Derived Micro-Organospheres from various sources into a receptacle prior to assaying.~~

30. ~~The method of any of claims 23-29, further comprising applying the Patient-Derived Micro-Organospheres into a receptacle comprising a plurality of chambers separated by an at least partially permeable membrane to permit circulation of supernatant material between the chambers.~~

31. ~~The method of any of claims 23-30, further comprising recovering one or more of the Patient-Derived Micro-Organospheres following the assay for further assaying or expansion.~~

32. ~~The method of any of claims 23-31, wherein assaying the plurality of Patient-Derived Micro-Organospheres comprises sampling one or more of a supernatant, an environment, and a microenvironment of the Patient-Derived Micro-Organospheres for secreted factors and other effects.~~

33. ~~The method of any of claims 23-32, wherein the tissue sample comprises cells that are not stem cells.~~

34. ~~The method of any of claims 23-33, wherein the tissue sample comprises a biopsy sample from a metastatic tumor.~~

35. ~~The method of any of claims 23-33, wherein the tissue sample comprises a clinical tumor sample, further wherein the clinical tumor sample comprises both cancer cells and stroma cells.~~

36. ~~The method of any of claims 23-33, wherein the tissue sample comprise tumor cells and one or more of: mesenchymal cells, endothelial cells, and immune cells.~~

37. ~~The method of any of claims 23-36, wherein combining the dissociated tissue sample and the fluid matrix material comprises disbursing the dissociated tissue sample within the fluid matrix material to a density of less than 5×10^6 cells/ml.~~

38. ~~The method of claims 23 or 24, wherein forming the droplet comprises forming the droplet from a continuous stream of the unpolymerized mixture.~~

39. The method of claim 38, wherein forming the droplet comprises applying one or more convergent streams of a fluid that is immiscible with the unpolymerized mixture to the stream of unpolymerized mixture.
40. The method of claims 23 or 24, wherein forming the droplet comprises forming the droplet in a fluid that is immiscible with the unpolymerized mixture.
41. The method of any of claims 25, 39 or 40, further comprising removing the immiscible fluid from the Patient-Derived Micro-Organospheres before culturing.
42. The method of any of claims 23-41, further comprising culturing the Patient-Derived Micro-Organospheres for between 2-14 days before assaying.
43. The method of claim 42, wherein culturing the Patient-Derived Micro-Organospheres comprises culturing the Patient-Derived Micro-Organospheres in suspension.
44. The method of any of claims 23-43, wherein combining the dissociated tissue sample and the fluid matrix material comprises combining the dissociated tissue sample with a basement membrane matrix.
45. The method of any of claims 23-44, wherein the tissue sample is combined with the fluid matrix material within six hours of removing the tissue sample from the patient.
46. The method of any of claims 23-45, further comprising genomically, transcriptomically, epigenomically and/or metabolically analyzing the cells in the Patient-Derived Micro-Organospheres before and/or after assaying or cryopreserving the Patient-Derived Micro-Organospheres.
47. The method of any of claims 23-46, further comprising dispensing the Patient-Derived Micro-Organospheres into a multi-well plate prior to assaying the Patient-Derived Micro-Organospheres.
48. The method of any of claims 23-47, wherein polymerizing comprises heating the droplet to greater than 35 degrees C.
49. The method of any of claims 23-48, wherein assaying the Patient-Derived Micro-Organospheres comprises exposing the Patient-Derived Micro-Organospheres to a drug.
50. The method of claims 23-49, wherein assaying comprises visually assaying the effect of the one or more agents on the cells in the Patient-Derived Micro-Organospheres.

51. The method of any of claims 23-50, wherein assaying comprises fluorescently assaying the effect of the one or more agents on the cells.

52. A composition of matter comprising a plurality of cryopreserved Patient-Derived Micro-Organospheres, wherein each Patient-Derived Micro-Organosphere has a spherical shape having a diameter of between 50 μ m and 500 μ m and comprises a polymerized base material, and between about 1 and 1000 dissociated primary cells distributed within the base material that have been passaged less than six times, whereby heterogeneity of the cells within the Patient-Derived Micro-Organospheres is maintained.

53. A composition of matter comprising a plurality of cryopreserved Patient-Derived Micro-Organospheres, wherein each Patient-Derived Micro-Organosphere has a spherical shape having a diameter of between 50 μ m and 500 μ m, wherein the Patient-Derived Micro-Organospheres have less than a 25% variation in size, and wherein each Patient-Derived Micro-Organosphere comprises a polymerized base material, and between about 1 and 500 dissociated primary cells distributed within the base material that have been passaged less than six times, whereby heterogeneity of the cells within the Patient-Derived Micro-Organospheres is maintained.

54. The composition of claims 52 or 53, wherein the primary cells are primary tumor cells.

55. The composition of any of claims 52-54, wherein the dissociated primary cells have been genetically or biochemically modified.

56. The composition of claim 52, wherein the plurality of cryopreserved Patient-Derived Micro-Organospheres have a uniform size with less than 25% variation in size.

57. The composition of any of claims 52-56, wherein the plurality of cryopreserved Patient-Derived Micro-Organospheres comprise Patient-Derived Micro-Organospheres from various sources.

58. The composition of any of claims 52-57, wherein the majority of cells in each Micro-Organosphere comprise cells that are not stem cells.

59. The composition of any of claims 52-58, wherein the primary cells comprises metastatic tumor cells.

60. The composition of any of claims 52-58, wherein the primary cells comprises both cancer cells and stroma cells.

61. The composition of any of claims 52-58, wherein the primary cells comprises tumor cells and one or more of: mesenchymal cells, endothelial cells, and immune cells.
62. The composition of any of claims 52-61, wherein the primary cells distributed within the polymerized base material are distributed at a density of less than 5×10^6 cells/ml.
- 5 63. The composition of any of claims 52-62, wherein the polymerized base material comprises a basement membrane matrix.
64. The composition of any of claims 52-63 wherein the polymerized base material comprises a synthetic material.
- 10 65. The composition of any of claims 52-64, wherein the microorganoids have a diameter of between 100 μm and 400 μm .
66. The composition of any of claims 52-65, wherein there are between 1-200 cells per Patient Derived Micro Organosphere.
- 15 67. A method of operating a Patient Derived Micro Organosphere forming apparatus, the method comprising:
- receiving an unpolymerized mixture comprising a chilled mixture of a dissociated tissue sample and a first fluid matrix material in a first port;
- receiving a second fluid that is immiscible with the unpolymerized mixture in a second port;
- 20 combining a stream of the unpolymerized mixture with one or more streams of the second fluid to form droplets of the unpolymerized mixture having a uniform size that varies by less than 25%; and
- polymerizing the droplets of the unpolymerized mixture to form a plurality of Patient Derived Micro Organospheres.
- 25 68. A method of operating a Patient Derived Micro Organosphere forming apparatus, the method comprising:
- receiving an unpolymerized mixture comprising a chilled mixture of a dissociated tissue sample and a first fluid matrix material in a first port;
- receiving a second fluid that is immiscible with the unpolymerized mixture in a second port;
- 30 combining a stream of the unpolymerized mixture at a first rate with one or more streams of the second fluid at a second rate to form droplets of the unpolymerized

~~mixture having a uniform size that varies by less than 25%, wherein the droplets are between 50 μ m and 500 μ m diameter; and
polymerizing the droplets of the unpolymerized mixture to form a plurality of Patient-Derived Micro-Organospheres.~~

5 ~~69. The method of claim 67 or 68, further comprising coupling a first reservoir containing the unpolymerized mixture in fluid communication with the first port.~~

~~70. The method of any of claims 67-69, further comprising combining the dissociated tissue sample and the first fluid matrix material to form the unpolymerized mixture.~~

10 ~~71. The method of any of claims 67-70, further comprising adding the unpolymerized mixture to a first reservoir in fluid communication with the first port.~~

~~72. The method of any of claims 67-71, further comprising coupling a second reservoir containing the second fluid in fluid communication with the second port.~~

~~73. The method of any of claims 67-72, further comprising adding the second fluid to a second reservoir in fluid communication with the second port.~~

15 ~~74. The method of any of claims 67-73, further comprising separating the second fluid from the plurality of Patient-Derived Micro-Organospheres.~~

~~75. The method of any of claims 67-74, wherein the dissociated tissue sample comprises primary cells.~~

20 ~~76. The method of any of claims 67-75, wherein the dissociated tissue sample comprises primary tumor cells.~~

~~77. The method of any of claims 67-75 wherein the dissociated tissue sample comprises metastatic tumor cells.~~

~~78. The method of any of claims 67-75, wherein the dissociated tissue sample comprises both cancer cells and stroma cells.~~

25 ~~79. The method of any of claims 67-75, wherein the dissociated tissue sample comprises tumor cells and one or more of: mesenchymal cells, endothelial cells, and immune cells.~~

~~80. The method of any of claims 67-79, wherein the fluid matrix material comprises a basement membrane matrix material.~~

~~81. The method of any of claims 67-79, wherein the fluid matrix material comprises a synthetic material.~~

~~82. The method of any of claims 67-79, wherein receiving the second fluid comprises receiving an oil.~~

~~83. The method of any of claim 67, wherein combining the streams comprises driving the stream of the unpolymerized mixture at a first flow rate across one or more streams of the second fluid which is traveling a second flow rate.~~

~~84. The method of claim 83, wherein the first flow rate is greater than the second flow rate.~~

~~85. The method of any of claims 67-84, wherein combining the streams comprises driving the stream of the unpolymerized mixture across a junction into which the one or more streams of the second fluid also converge.~~

~~86. The method of any of claims 67-85, wherein polymerizing the droplets comprises heating the droplets to greater than 30 degrees C.~~

~~87. The method of any of claims 67-86, further comprising aliquoting the plurality of Patient-Derived Micro-Organospheres.~~

~~88. The method of claim 87, wherein aliquoting comprises aliquoting into a multi-well dish.~~

~~89. A method comprising:~~

~~receiving a patient tumor tissue;~~

~~determining, within 2 weeks of taking of the tumor tissue from the patient, that the tumor will respond to a drug formulation by: forming, from the patient tumor tissue, a plurality of microoganoids having a diameter of between 50 and 500 μ m with between 1 and 200 dissociated tumor cells distributed through a polymerized base material, and exposing at least some of the Patient-Derived Micro-Organospheres to the drug formulation before the dissociated tumor cells have undergone more than five passages; and~~

~~measuring an effect of the drug formulation on the cells within the at least some of the microoranoids to determine if the drug will treat the tumor based on the determined effect.~~

- 5 90. ~~The method of claim 89, further comprising determining that the tumor is still responding to the drug formulation after one or more administrations of the drug by receiving a second patient tumor tissue after the patient has been treated with the drug formulation and forming a second plurality of Patient-Derived Micro-Organospheres from the second patient tumor tissue, exposing at least some of the second plurality of Patient-Derived Micro-Organospheres to the drug formulation, and measuring the effect of the drug formulation on cells within the at least some of the second plurality of microorganoids.~~
- 10 91. ~~The method of claim 89, wherein determining that the tumor will respond to a drug formulation comprises exposing at least some of the Patient-Derived Micro-Organospheres to a plurality of drug formulations, and reporting the measured effects for each of the drug formulations.~~
- 15 92. ~~The method of claim 89, wherein determining further comprises dispensing the microorganoids into a multi-well plate prior to assaying the Patient-Derived Micro-Organosphere.~~
- 20 93. ~~The method of claim 89, further comprising biopsying the patient to collect the patient tumor tissue.~~
94. ~~The method of claim 89, further comprising treating the patient with the drug formulation.~~
- 25 95. ~~The method of claim 89, wherein the time between receiving the tumor tissue and reporting is less than 21 days.~~
96. ~~The method of claim 89, wherein patient tumor tissue comprises a biopsy sample from a metastatic tumor.~~
97. ~~The method of claim 89, wherein the patient tumor tissue comprises both cancer cells and stroma cells.~~
98. ~~The method of claim 89, wherein the patient tumor tissue comprise tumor cells and one or more of: mesenchymal cells, endothelial cells, and immune cells.~~
99. ~~The method of claim 89, wherein forming the plurality of microorganoids comprising forming microorganoids having less than a 25% variation in size.~~