

[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

[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:
forming a plurality of droplets of an unpolymerized mixture from a dissociated tissue sample and a fluid matrix material; and
5 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. The method of claim 1, further comprising modifying the cells within the dissociated tissue sample prior to forming the droplets.
- 10 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.
4. The method of claim 1, wherein the tissue sample comprises cells that are not stem cells.
5. The method of claim 1, wherein the tissue sample comprises a biopsy sample from a
15 metastatic tumor.
6. The method of claim 1, wherein the tissue sample comprises a clinical tumor sample, further wherein the clinical tumor sample comprises both cancer cells and stroma cells.
7. The method of claim 1, wherein the tissue sample comprise tumor cells and one or more of: mesenchymal cells, endothelial cells, and immune cells.
- 20 8. The method of claim 1, 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.
9. The method of claim 8, further comprising removing the immiscible fluid from the Patient-Derived Micro-Organosphere.
- 25 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 claim 1, wherein combining the dissociated tissue sample and the fluid matrix material comprises combining the dissociated tissue sample with a MATRIGEL substrate basement membrane matrix.
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.
20. 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,

- 5 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.