

patient's own immune cells (e.g., T cells, NK cells, B cells, etc.) to attack their tumors, the effectiveness of immunotherapy is not equivalent for all patients or cancer types, which has led to the need for better models for scientists and researchers.

[0004] Accordingly, on-going need exists for alternative models and methods to enable the investigation immune cell and drug homing, tumor cytotoxicity, and tumor immune evasion in a single, easy-to-use, high throughput system for more *in vivo*-like testing.

SUMMARY

[0005] In accordance with various examples of the present disclosure, methods and labware for assaying therapeutic agents, including immune cells and drugs, and their effects on tumor cells growing in 3D spheroid conformation in culture are disclosed herein. The methods allow for, among other uses, the investigation of the effects of a therapeutic agent, such as immune cells or a drug, on tumor cells, and enable the investigation of homing, tumor cytotoxicity, and tumor immune evasion in a single, easy-to-use, high throughput system for more *in vivo*-like testing.

[0006] In one aspect, the present disclosure refers to an assay method for detecting active migration and cytotoxicity of a therapeutic agent is disclosed. The assay method includes culturing tumor cells in a cell culture article to form a spheroid, wherein the cell culture article comprises a chamber structured to constrain the tumor cells to grow in a 3D spheroid conformation. The assay method further includes placing an insert comprising a porous membrane into the cell culture article and introducing a therapeutic agent into the insert. The assay method also includes detecting active migration of the therapeutic agent from the insert into the cell culture article chamber and detecting tumor cell response. In examples, tumor cell response may be tumor cell lysis, infiltration of the therapeutic agent into the tumor cell spheroid, or measurements of changes in tumor cell physiology.

[0007] In some examples, both active migration of the therapeutic agent and tumor cell lysis are detected by flow cytometry. In some examples, the assay method further includes detecting infiltration of the therapeutic agent, such an immune cell, into the tumor cell spheroid.

[0008] In some examples, the therapeutic agent is a cell therapeutic and/or a drug. In some examples, the cell therapeutic includes an immune cell. In some examples, the immune cell is a leukocyte. In some examples, the immune cell is a lymphocyte.

[0009] In some examples of an assay method for detecting active migration and cytotoxicity of a therapeutic agent, the chamber of the cell culture article includes a side wall, a top aperture, and a liquid impermeable bottom comprising at least one concave surface. In examples, at least a portion of the bottom surface includes a low-adhesion or no-adhesion material in or on the at least one concave surface. In some examples, the liquid impermeable bottom including at least one concave surface is gas-permeable. In some examples, the side wall is opaque. In some examples, at least a portion of the bottom is transparent.

[0010] In some examples of an assay method for detecting active migration and cytotoxicity of a therapeutic agent, the cell culture article includes from 1 to about 2,000 of said chambers, wherein each chamber is physically separated from any other chamber. In some examples, the at least one concave surface of the chamber includes a plurality of concave surfaces within the same chamber.

[0011] In some examples of an assay method for detecting active migration and cytotoxicity of a therapeutic agent, the at least one concave surface the chamber of the cell culture article includes a hemi-spherical surface, a conical surface having a taper of 30 to about 60 degrees from the side walls to the bottom surface, or a combination thereof.

[0012] In some examples of an assay method for detecting active migration and cytotoxicity of a therapeutic agent, the side wall surface of the chamber of the cell culture article includes a vertical cylinder, a portion of a vertical conic of decreasing diameter from the chamber's top to bottom surface, a vertical square shaft having a conical transition to the at least one concave bottom surface, or a combination thereof.

[0013] In some examples of an assay method for detecting active migration and cytotoxicity of a therapeutic agent, the cell culture article further includes a chamber annex for receiving a pipette tip for aspiration, the chamber annex including a surface adjacent to and in fluid communication with the chamber, the chamber annex having a second bottom spaced away and at an elevation above the bottom surface, wherein the second bottom deflects fluid dispensed from a pipette away from the bottom surface.

[0014] In some examples of an assay method for detecting active migration and cytotoxicity of a therapeutic agent, the insert includes an insert plate.

[0015] In some examples of an assay method for detecting active migration and cytotoxicity of a therapeutic agent, at least a portion of the porous membrane is configured to simulate a biological barrier. In some examples, the biological barrier is a blood-brain barrier. In some examples, at least a portion of the porous membrane includes an essentially confluent monolayer of microvascular endothelial cells.

[0016] Additional features and advantages of the subject matter of the present disclosure will be set forth in the detailed description which follows, and in part will be readily apparent to those skilled in the art from that description or recognized by practicing the subject matter of the present disclosure as described herein, including the detailed description which follows, the claims, as well as the appended drawings.

[0017] It is to be understood that both the foregoing general description and the following detailed description present embodiments of the subject matter of the present disclosure, and are intended to provide an overview or framework for understanding the nature and character of the subject matter of the present disclosure as it is claimed. The accompanying drawings are included to provide a further understanding of the subject matter of the present disclosure, and are incorporated into and constitute a part of this specification. The drawings illustrate various embodiments of the subject matter of the present disclosure and together with the description serve to explain the principles and operations of the subject matter of the present disclosure. Additionally, the drawings and descriptions are meant to be merely illustrative, and are not intended to limit the scope of the claims in any manner.

DESCRIPTION OF THE FIGURES

[0018] The following detailed description of specific embodiments of the present disclosure can be best understood when read in conjunction with the following drawings, where like structure is indicated with like reference numerals and in which:

[0019] **FIG. 1A, FIG. 1B and FIG. 1C** show an embodiment of a multi-well microplate, in this case a 96-well spheroid microplate, having an array of microcavities on the bottom surface of each well to provide multiple spheroids in each of the 96 wells. **FIG. 1A** shows a multi-well microplate. **FIG. 1B** shows illustrates a single well of the multi-well plate. **FIG.**

CLAIMS

What is claimed is:

1. An assay method for detecting active migration and cytotoxicity of a therapeutic agent, comprising:
 - a) culturing tumor cells in a cell culture article to form a spheroid, wherein the cell culture article comprises a chamber structured to constrain the tumor cells to grow in a 3D spheroid conformation;
 - b) placing an insert comprising a porous membrane into the cell culture article and introducing a therapeutic agent into the insert;
 - c) detecting active migration of the therapeutic agent from the insert into the cell culture article chamber; and
 - d) detecting tumor cell response.
2. The assay method of claim 1, wherein the chamber comprises:
 - a side wall;
 - a top aperture; and
 - a liquid impermeable bottom comprising at least one concave surface, wherein at least a portion of the bottom comprises a low-adhesion or no-adhesion material in or on the at least one concave surface.
3. The assay method of claim 1 or 2, wherein the liquid impermeable bottom comprising at least one concave surface is gas-permeable.
4. The assay method of claim 2, wherein the side walls are opaque.

5. The assay method of any one of claims 2-4, wherein at least a portion of the bottom is transparent.
6. The assay method of any one of claims 2-5, wherein the cell culture article comprises from 1 to 2,000 of said chambers, wherein each chamber is physically separated from any other chamber.
7. The assay method of any one of claims 2-6, wherein the at least one concave surfaces comprises a plurality of concave surfaces within the same chamber.
8. The assay method of any one of claims 2-7, wherein the at least one concave surface comprises a hemi-spherical surface, a conical surface having a taper of 30 to 60 degrees from the side walls to the bottom surface, or a combination thereof.
9. The assay method of claim 2, wherein the side wall surface comprises a vertical cylinder, a portion of a vertical conic of decreasing diameter from the chamber's top to bottom surface, a vertical square shaft having a conical transition to the at least one concave bottom surface, or a combination thereof.
10. The assay method of claim 2, the article further comprising a chamber annex for receiving a pipette tip for aspiration, the chamber annex comprising a surface adjacent to and in fluid communication with the chamber, the chamber annex having a second bottom spaced away and at an elevation above the bottom surface, wherein the second bottom deflects fluid dispensed from a pipette away from the bottom surface.
11. The assay method of any one of claims 1-10, wherein the insert comprises an insert plate.
12. The assay method of any one of claims 1-11, wherein at least a portion of the porous membrane is configured to simulate a blood-brain barrier.

13. The assay method of claim 12, wherein at least a portion of the porous membrane comprises an essentially confluent monolayer of microvascular endothelial cells.
14. The assay method of any one of claims 1-13, wherein both active migration of the therapeutic agent and tumor cell response are detected by flow cytometry.
15. The assay method of any one of claims 1-14, wherein detecting tumor cell response comprises detecting infiltration of the therapeutic agent into the tumor cell spheroid.
16. The assay method of any one of claims 1-15, wherein detecting tumor cell response comprises measuring tumor cell lysis.
17. The assay method of any one of claims 1-16, wherein the therapeutic agent comprises a drug or a cell therapeutic.
18. The assay method of claim 17, wherein the therapeutic agent comprises a leukocyte or a lymphocyte.
19. The assay method of any one of claims 10-18, wherein the therapeutic agent is a drug.