# SPHEROID TRAP INSERT

## PRIORITY CLAIM

The present application claims priority to U.S. Provisional Application serial number 62/072094, filed October 29, 2014, which is herein incorporated by reference.

## FIELD

**[0001]** The present disclosure relates to apparatuses, systems and methods for culturing cells.

## TECHNICAL BACKGROUND

**[0002]** Cells cultured in three dimensions, such as spheroids, can exhibit more *in-vivo* like functionality than their counterparts cultured in two dimensions as monolayers. In two dimensional cell culture systems, cells can attach to a substrate on which they are cultured. However, when cells are grown in three dimensions, such as spheroids, the cells interact with each other rather than attaching to the substrate. Accordingly, cell culture media exchanges for culture systems in which cells are cultured as spheroids that are mobile in suspension can present challenges.

## BRIEF SUMMARY

**[0003]** In accordance with various embodiments of the present disclosure, cell culture inserts configured to allow exchange of culture media in wells containing spheroids without aspirating or damaging the spheroids are described. The cell culture inserts can include a frame having a first open end, a second open end, and at least one support extending between the first open end and the second open end. The inserts also include a fluid permeable mesh coupled to the frame and disposed across the opening of the second end. The mesh defines pores having an average pore size in a range from 10 micrometers to 200micrometers, e.g., 10, 20, 50, 100 or 200 micrometers, including ranges between any of the foregoing values. In embodiments, the pores are sized to allow individual cells to pass across or through the mesh, but to prevent spheroid cell clusters from passing.

**[0003a]** In an aspect, there is provided a cell culture assembly comprising:

a cell culture container comprising at least one well having an opening and a nadir; an insert structured to fit inside the at least one well of the cell culture container, said insert comprising a first open end, a second end having an opening, wherein said first

open end and said second end define a cavity therebetween,

wherein the second end comprises a fluid permeable mesh, wherein the cell culture container comprises a cell culture surface and wherein said cell culture surface comprises a substrate having an array of microwells.

**[0003b]** In another aspect, there is provided a method of culturing spheroid cells in a cell culture assembly as defined above comprising:

positioning an insert in a well;

introducing cells and media into the cavity of the insert,

allowing cells to pass through the mesh of the insert and form spheroids in the space between the nadir of the well and the mesh of the insert.

**[0003c]** In another aspect, there is provided a method of removing media from a cell culture assembly as defined above comprising:

positioning an insert in a well;

introducing cells and media into the cavity of the insert,

allowing cells to pass through the mesh of the insert and form spheroids in the space between the nadir of the well and the mesh of the insert

removing the media from the well above the insert.

**[0003d]** In another aspect, there is provided a cell culture assembly comprising:

a well comprising an aperture, sidewalls and a nadir;

wherein the nadir comprises an array of microwells;

an insert comprising:

an aperture, a frame and a fluid permeable mesh comprising pores,

wherein the pores comprise an average pore size in a range from 10 micrometers to 100 micrometers, and wherein the insert is configured to be positioned within at least a portion of the well at a distance from the nadir.

**[0003e]** In another aspect, there is provided a method of culturing spheroid cells in a cell culture assembly as defined above comprising:

positioning an insert in a well;

introducing cells and media into the cavity of the insert,

allowing cells to pass through the mesh of the insert and form spheroids in the space between the nadir of the well and the mesh of the insert.

**[0003f]** In another aspect, there is provided a method of removing media from a cell culture

assembly as defined above comprising:

positioning an insert in a well;

introducing cells and media into the cavity of the insert,

allowing cells to pass through the mesh of the insert and form spheroids in the space between the nadir of the well and the mesh of the insert

removing the media from the well above the insert.

**[0004]** In some embodiments, provided herein are cell culture well inserts comprising: a frame having a first open end, a second end defining an opening, and at least one support extending between the first open end and the second end, wherein the opening