

## 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 200 micrometers, 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;  
wherein the nadir comprises a substrate having an array of microwells; 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, and  
wherein the second end comprises a fluid permeable mesh.

[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 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.

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

What is claimed is:

1. A cell culture assembly comprising:  
a cell culture container comprising at least one well having an opening and a nadir;  
wherein the nadir comprises a substrate having an array of microwells; 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, and  
wherein the second end comprises a fluid permeable mesh.
2. The cell culture assembly of claim 1, wherein the mesh comprises pores having an  
average pore size in a range from 10 micrometers to 200 micrometers.
3. A cell culture assembly according to claim 1 or 2, wherein the well is configured such  
that cells cultured in the well form spheroid in the array of microwells.
4. A cell culture assembly according to claim 3, wherein the pores of the mesh are of a size  
that allows passage of individual cells through the mesh and prevents passage of spheroids  
formed in the array of microwells through the mesh.
5. The cell culture assembly of any one of claims 1-4, wherein well insert further  
comprises a side wall, or a plurality of supports, extending from the first end to the second  
end.
6. The cell culture insert of claim 5, wherein at least a portion of said side wall  
comprises said mesh.

15. The cell culture assembly according to any of claims 1 to 12,  
wherein the insert further comprises  
a frame, wherein the fluid permeable mesh comprises pores; and  
wherein the pores comprise an average pore size in a range from 10 micrometers  
to 100 micrometers.
16. The cell culture assembly of claim 15, wherein the pores of the mesh are configured to  
prevent spheroids from passing through the mesh.
17. The cell culture assembly according to claim 15 or 16, wherein the well further defines  
an upper edge, wherein the frame contacts the upper edge to position the mesh a  
distance from the nadir.
18. The cell culture assembly according to claim 15 or 16, further comprising a support,  
wherein the frame contacts the support to position the mesh a distance from the nadir.
19. The cell culture assembly according to any one of claims 15 to 18, wherein interior  
surface of the well comprises a conical shape from the aperture to the nadir.
20. The cell culture assembly according to any one of claims 15 to 19, wherein the mesh  
comprises a circular shape.

21. The cell culture assembly according to any one of claims 15 to 19, wherein the mesh defines a square shape.
22. The cell culture assembly according to any one of claims 15 to 21, further comprising a plurality of wells and wherein the insert comprises a plurality of inserts, wherein each of the inserts is configured to be positioned in at least a portion of a different one of the wells of the plurality of wells.
23. The cell culture assembly according to any one of claims 15 to 22, wherein the interior surface of the well is coated with an ultra-low-adhesion material.
24. The cell culture assembly according to any one of claims 15 to 23, wherein at least a portion of the frame comprises mesh.
25. The cell culture assembly according to any one of claims 15 to 24, wherein the frame comprises at least one support wire coupled to the fluid permeable mesh.
26. The cell culture assembly according to any one of claims 15 to 25, wherein the well comprises at least one sub-well along the interior surface.
27. A method of culturing spheroid cells in a cell culture assembly of any one of claims 15-26 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.

28. A method of removing media from a cell culture assembly of any one of claims

15-26 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.