Claims

1. A multi-layer microfluidic bioreactor for cell culture, comprising a header layer, a base layer and a fluid permeable flow layer between the header layer and the base layer, wherein:

(a) the flow layer comprises an upper surface and a lower surface, and the upper surface comprises one or more grooves for retention of cells;

(b) the base layer provides a gas flow path for gas exchange across the flow layer; and

(c) the header layer is configured to define a flow channel over the flow layer upper surface, and the header layer comprises:

(i) a first inlet port;

(ii) a first outlet port;

(iii) one or more first fluid inlets providing fluid communication between the first inlet port and the flow channel; and

(iv) one or more first fluid outlets providing fluid communication between the flow channel and the first outlet port;

wherein the one or more first fluid inlets and the one or more first fluid outlets are positioned in the header layer such that fluid entering the flow channel from the one or more first fluid inlets moves in a first flow direction toward the one or more first fluid outlets, the first flow direction being across the one or more grooves to minimise disruption of cells received therein.

2. The multi-layer microfluidic bioreactor of claim 1, wherein the header layer comprises:

- a second outlet port; and

- one or more second fluid outlets providing fluid communication between the flow channel and the second outlet port;

wherein the one or more second fluid outlets are positioned in the header layer such that fluid exiting the flow channel through the second outlet port flows in a second flow direction toward the one or more second fluid outlets, the second flow direction being along the one or more grooves.

3. The multi-layer microfluidic bioreactor according to claim 2, wherein the header layer further comprises:
- a second inlet port; and
- one or more second fluid inlets providing fluid communication between the second inlet port and the flow channel;

wherein the one or more second fluid inlets are positioned along a dimension of the header which is substantially orthogonal to a longitudinal dimension of the one or more grooves so that fluid entering the flow channel from the one or more second fluid inlets flows toward the one or more second fluid outlets in the second flow direction.

4. The multi-layer microfluidic bioreactor according to claim 3, wherein the second inlet port receives fluid from one or more media sources, and the header layer comprises one or more second fluid channels for receiving the fluid media into the second inlet port, and optionally, wherein the one or more second fluid channels extend, preferably colinearly, through the header layer, the flow layer and the base layer, to form vertical channels through the multilayer microfluidic bioreactor.

5. The multi-layer microfluidic bioreactor according to any one of claims 2 to 4, wherein the first flow direction and the second flow direction are substantially orthogonal.

6. The multi-layer microfluidic bioreactor according to any one of claims 1 to 5, further comprising a selectively permeable membrane separating the flow channel into a first flow channel and a second flow channel; wherein the selectively permeable membrane provides for exchange of ~~low molecular weight~~ metabolites having molecular weight less than substantially 10,000 Daltons.

7. The multi-layer microfluidic bioreactor according to claim 6, further comprising a secondary header layer, wherein the selectively permeable membrane is between the header layer and the secondary header layer thereby forming the second flow channel therebetween, wherein the secondary header layer

comprises:

(i) a secondary inlet port in fluid communication with the second flow channel; and optionally,

(ii) a secondary outlet port in fluid communication with the second flow channel;

wherein the selectively permeable membrane provides for exchange of low molecular weight fluid metabolites entering the secondary inlet port and optionally, for removal of low molecular weight fluid metabolites through the secondary outlet port~~.~~;

wherein the low molecular weight fluid metabolites have a molecular weight less than substantially 10,000 Daltons.

8. The multi-layer microfluidic bioreactor according to any one of claims 1 to 7, wherein the one or more grooves are functionalised to limit non-specific binding of cells in the one or more grooves.

9. The multi-layer microfluidic bioreactor according to any one of claims 1 to 8, wherein the one or more grooves are functionalised with a capture reagent that selectively captures cells.

10. The multi-layer microfluidic bioreactor according to any one of claims 1 to 9, wherein the one or more grooves are functionalised with polymers that limit non-specific binding of cells in the one or more grooves.

11. The multi-layer microfluidic bioreactor according to any one of claims 1 to 10, configured for arrangement in a stack including at least one further multi-layer microfluidic bioreactor, each multi-layer microfluidic bioreactor comprising one or more through ports extending colinearly through each of the header layer, the base layer and the flow layer and providing fluid communication with corresponding through ports of the at least one further multi-layer microfluidic bioreactor, and optionally further comprising one or more alignment features for positioning of at least one further multi-layer microfluidic bioreactor in stacked arrangement.

12. A bioreactor cell culture system comprising:

(a) a plurality of multi-layer microfluidic bioreactors according to any one of claims 1 to 11 arranged in at least one stack;

(b) a controller configured to control individually operation of one or more fluid pumps according to a cell culture protocol stored in a memory of the controller, the one or more fluid pumps delivering fluid to one or more through ports provided colinearly through the at least one stack, the delivered fluid entering the flow channel of each microfluidic bioreactor in the at least one stack according to the stored cell culture protocol.

13. The bioreactor cell culture system of claim 12, comprising one or both of:

(a) a substantially rigid base plate beneath the at least one stack; and

(b) a substantially rigid cover plate arranged over the at least one stack;

wherein each of the base plate and the cover plate impart structural support to the at least one stack, and optionally, wherein the substantially rigid cover plate comprises a plurality of openings providing fluid communication between through ports in the one or more stacks and one or more fluid sources and/or waste reservoirs.

14. The bioreactor cell culture system according to any one of claims 12 to 13, wherein the controller is operable to cause extraction of cells from the one or more grooves of the individual multi-layer microfluidic bioreactors arranged in a stack through a cell extraction through port extending colinearly through the stack of multi-layer microfluidic bioreactors, and in fluid communication with the second outlet port in the individual multi-layer microfluidic bioreactors, and optionally further comprising a coupling for receiving a receptacle for uncontaminated collection of extracted cells from the one or more stacks of microfluidic bioreactors.

15. A multi-layer bioreactor sheet comprising a plurality of multi-layer microfluidic bioreactors, each of the multi-layer microfluidic bioreactors comprising a culture chamber containing one or more grooves and one or more through ports providing for flow of fluid across the one or more grooves, wherein the

one or more through ports are provided colinearly through the layers of multi-layer the bioreactor sheet.

16. A multi-layer bioreactor sheet according to claim 15, wherein each multi-layer bioreactor sheet is configured for arrangement into a sheet stack comprising at least one other multi-layer bioreactor sheet, wherein through ports in each multi-layer bioreactor sheet in the sheet stack extend colinearly providing fluid communication with the culture chambers of individual multi-layer microfluidic bioreactors in each multi-layer bioreactor sheet.

17. A bioreactor cell culture system for use with one or more multi-layer bioreactor sheets arranged in a sheet stack according to claim 16, the system comprising:

(a) a controller to control individually, operation of one or more fluid pumps according to a cell culture protocol stored in a memory of the controller;

(b) a sheet stack header providing fluid coupling between at least one fluid or waste reservoir in the bioreactor cell culture system and multiple through ports in the sheet stack requiring flow to or from the at least one fluid or waste reservoir according to the stored cell culture protocol; and

(c) one or more fluid pumps delivering fluid via the through ports to individual culture chambers of the multi-layer microfluidic bioreactors in accordance with the stored cell culture protocol.

18. Use of a multi-layer microfluidic bioreactor according to any one of claims 1 to 11, or a multi-layer bioreactor sheet according to claim 15 or 16, or a bioreactor cell culture system according to any one of claims 12, 13, 14 and ~~to~~ 17 for cell culture, cell transfection/transduction, or cell selection.