

CLAIMS

1. A microfluidic device comprising:

at least one well for cultivating cells, the well having an inlet and an outlet,
5 wherein the cross-sectional area of the outlet is smaller than the cross-sectional area of the inlet;

an influent channel for perfusing medium into the well, the influent channel being in fluid communication with the inlet of the well; and

an effluent channel for carrying away fluid from the well, the effluent channel
10 being in fluid communication with the outlet of the well, wherein

the influent channel is inclined such that the flow of the medium along an inclined portion of the influent channel is against gravitational force during medium perfusion in an upright position, the upright position being a position where the influent channel is above the well and the direction of perfusion of medium into the well is
15 substantially parallel to the direction of gravity.

2. The microfluidic device of claim 1, wherein at least a portion of the well tapers towards the outlet.

20 3. The microfluidic device of claim 1, wherein the influent channel is inclined at an angle relative to a bottom edge of the upright microfluidic device for allowing gas bubbles to exit from the microfluidic device.

4. The microfluidic device of claim 3, wherein the effluent channel is
25 inclined at an angle relative to a bottom edge of the microfluidic device for allowing gas bubbles to exit from the microfluidic device.

5. The microfluidic device of claim 4, wherein the angle of inclination of the effluent channel is substantially the same as the angle of inclination of the influent
30 channel.

6. The microfluidic device of claim 1, wherein the well is an elongate well configured to allow substantially uniform and non-turbulent laminar flow of liquid from the influent channel into the well.

5 7. The microfluidic device of claim 6, wherein the cross-section of the elongate well has a shape with at least two vertices.

8. The microfluidic device of claim 7, wherein the cross-section of the elongate well is substantially rectangular in shape.

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9. The microfluidic device of claim 1, wherein the microfluidic device is configured to allow the surface tension force (ΔP_Y) at the outlet to be no less than the flow resistance (ΔP_D) of the influent channel when in use, and

15 wherein, the surface tension force (ΔP_Y) is calculated from the Young-Laplace equation (1)

$$\Delta P_Y = \gamma \left(\frac{1}{R_1} + \frac{1}{R_2} \right), \quad (1)$$

where γ is the surface tension of the filling liquid, and R_1 and R_2 are the principal radii of curvature, and the flow resistance (ΔP_D) is calculated from the Hagen-Poiseuille equation (2)

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$$\Delta P_D = \frac{128\mu L_c Q}{\pi d_c^4}. \quad (2)$$

where μ is the viscosity of the filling solution, L_c and d_c are the length and diameter of the influent channel, respectively, and Q is the flow rate along the influent channel.

25 10. The microfluidic device of claim 1, wherein the device comprises a plurality of wells and the influent channel is in fluid communication with the inlets of the plurality of wells, and the effluent channel is in fluid communication with the outlets of the plurality of wells and runs across said outlets.

11. The microfluidic device of claim 1, wherein the influent channel comprises:

5 the inclined portion inclined at an angle for allowing gas bubbles to exit from the microfluidic device;

one or more openings that is configured to be coupled to a connection tip for coupling to an external tubing for perfusion or for removal of gas bubbles; and

one or more connecting portions configured to provide fluid communication between the inclined portion and the one or more openings.

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12. The microfluidic device of claim 1, wherein the effluent channel comprises:

an inclined portion inclined at an angle for allowing gas bubbles to exit from the microfluidic device;

15 one or more openings that is configured to be coupled to a connection tip for coupling to an external tubing for removal of gas bubbles; and

one or more connecting portions configured to provide fluid communication between the inclined portion and the one or more openings.

20 13. The microfluidic device of claim 1, wherein the device further comprises a cover disposed over the well and channels for generating a substantially closed system.

25 14. The microfluidic device of claim 1, wherein the microfluidic device is an organ-on-chip device or a bioreactor or a microphysiologic system.

15. A method of introducing flowable contents in the microfluidic device of claim 10 in an upright position, the method comprising:

(i) introducing the flowable content through the influent channel;

(ii) allowing the flowable content to enter a first of the plurality of wells through the inlet of said well while optionally displacing existing gas from said well via the outlet to the effluent channel;

5 (iii) allowing the flowable content to fill the first of the wells such that the flowable content overflows from the first of the wells into the influent channel and the overflowing flowable content is transported to a second of the wells via the influent channel;

(iv) allowing the flowable content to enter the second of the wells through the inlet of said second well while optionally displacing existing gas from said second well
10 via the outlet to the effluent channel; and

(v) optionally repeating steps (ii) to (iv) for the third and subsequent wells.

16. The method of claim 15, wherein the method is adapted to allow substantially uniform cell seeding in the plurality of wells.
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17. The method of claim 15, wherein the method further comprises removing gas bubbles from the flowable content by allowing the gas bubbles to be transported away from the wells via the influent channel and/or effluent channels.

20 18. The method of claim 17, wherein one or more of the steps are performed when the microfluidic device is in an upright position, the upright position being a position where the direction of introduction of flowable content into the well is substantially parallel to the direction of gravity.

25 19. A method of manufacturing a microfluidic device comprising:
providing at least one well for cultivating cells, the well having an inlet and an outlet, wherein the cross-sectional area of the outlet is smaller than the cross-sectional area of the inlet;

30 providing an influent channel for perfusing medium into the well, the influent channel being in fluid communication with the inlet of the well; and

providing an effluent channel for carrying away fluid from the well, the effluent channel being in fluid communication with the outlet of the well, wherein

the influent channel is inclined such that the flow of the medium along an inclined portion of the influent channel is against gravitational force during medium perfusion in an upright position, the upright position being a position where the influent channel is above the well and the direction of perfusion of medium into the well is substantially parallel to the direction of gravity.

20. Use of the microfluidic device of claim 1 as an *in vitro* renal model for toxicity assessment of compounds in the physiological concentration range.

21. The use of the microfluidic device of claim 20, wherein the use comprises maintaining monolayers of cultured human primary renal proximal tubule cells (HPTCs) in the wells for at least 7 days.

22. The use of the microfluidic device of claim 21, wherein the monolayers of HPTCs comprise upregulated transporters and Cytochrome P450 (CYP) enzymes as compared to HPTCs cultured under static conditions for the same period of time.

23. The use of the microfluidic device of claim 20, wherein the use comprises repeated dose testing of a compound over a period of at least 7 days.

24. The use of the microfluidic device of claim 21, wherein the use comprises imaging HPTCs with subcellular resolution for determining toxicity endpoints.

25. The use of the microfluidic device of claim 21, wherein concentration-dependent cell death is inducible by at least 9 known nephrotoxics within the physiological concentration range.