

models of cardio-toxicity based on animal testing do not always correlate with clinical risks. As a result, disconnection between *in vitro* studies, translational animal models, and human clinical studies decreases the effectiveness of the resulting therapeutic strategies. Further, animal testing is a slow and expensive process and raises ethical concerns. Thus, there is a significant need for lower cost, easy-to-use, robust, high-throughput biological models that can be used to predict human safety and efficacy of a candidate drug or pharmaceutical substance.

SUMMARY

[0005] In one aspect of the present disclosure, there is provided a method of deriving and maintaining endothelial cells, comprising: (a) culturing lateral plate mesoderm cells under oxygen-deprived condition to obtain endothelial lineage cells; and (b) culturing the endothelial lineage cells from (a) on an extracellular matrix to maintain and expand the endothelial lineage cells; wherein the lateral plate mesoderm cells in (a) comprise splanchnic lateral plate mesoderm cells. Also described is a method of deriving and maintaining endothelial cells, the method comprising: (a) culturing lateral plate mesoderm cells under oxygen-deprived condition to obtain endothelial lineage cells; and (b) culturing the endothelial lineage cells from (a) on an extracellular matrix to maintain and expand the endothelial lineage cells.

[0006] In an example, there is provided a cell co-culture system comprising an endothelial cell culture and a hepatocyte cell culture.

[0007] In a further example, there is provided a microfluidic-based system comprising the cell co-culture system of the present invention.

[0008] In yet a further example, there is provide a method of disease modelling or drug testing using the cell co-culture system or the microfluidic-based system of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0010] **Fig. 1** shows the results of the generation and characterization of functional endothelial cells from human embryonic stem cells. **Fig. 1A** illustrates the timeline of endothelial induction from human pluripotent stem cells (hPSC), delineating the differentiation media and factors used. Human pluripotent stem cell-derived endothelial cells
5 (HPSC-ECs) were derived via a lateral plate mesoderm population on day 5. By day 10, platelet and endothelial cell adhesion molecule 1 (PECAM1)-expressing cells could be

Claims

1. A method of deriving and maintaining endothelial cells, comprising:
 - (a) culturing lateral plate mesoderm cells under oxygen-deprived condition to obtain
5 endothelial lineage cells; and
 - (b) culturing the endothelial lineage cells from (a) on an extracellular matrix to
maintain and expand the endothelial lineage cells;wherein the lateral plate mesoderm cells in (a) comprise splanchnic lateral plate
mesoderm cells.
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2. The method of claim 1, wherein the oxygen-deprived condition is carried out under
oxygen supply of between 0.5% to 5%, or at about 1%.
3. The method of any one of the preceding claims, wherein the lateral plate mesoderm
15 cells are cultured in a first cell culture medium, wherein the first cell culture medium
comprises:
 - (a) Fibroblast growth factor (FGF);
 - (b) TGF- β inhibitor; and
 - (c) Vascular endothelial growth factor (VEGF).
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4. The method of claim 3, wherein the lateral plate mesoderm cells are cultured in the
first cell culture medium for 3 to 7 days, or for about 5 days.
5. The method of claim 3 or 4, wherein the fibroblast growth factor is fibroblast growth
25 factor 2 (FGF2).
6. The method of any one of claims 3 to 5, wherein the fibroblast growth factor is at a
concentration of between 1 to about 10 ng/ml, or at about 4ng/ml.
- 30 7. The method of any one of claims 3 to 6, wherein the TGF- β inhibitor is 4-[4-(1,3-
benzodioxol-5-yl)-5-(2-pyridinyl)-1H-imidazol-2-yl]benzamide (SB 431542).

8. The method of any one of claims 3 to 7, wherein the TGF- β inhibitor is at a concentration of between 1 to 30 μ M, or at about 10 μ M.

9. The method of any one of claims 3 to 8, wherein the VEGF is VEGF-A.

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10. The method of claim 9, wherein the VEGF-A is of an isoform of VEGF₁₆₅.

11. The method of any one of claims 3 to 10, wherein the VEGF is at a concentration of between 10 to 100 ng/ml, or at about 50ng/ml.

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12. The method of any one of claims 3 to 11, wherein the fibroblast growth factor is fibroblast growth factor 2 (FGF2), the TGF- β inhibitor is 4-[4-(1,3-benzodioxol-5-yl)-5-(2-pyridinyl)-1H-imidazol-2-yl]benzamide (SB 431542), and the vascular endothelial growth factor is VEGF₁₆₅.

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13. The method of claim 12, wherein the FGF2 is at a concentration of between 1 to 10 ng/ml, or at about 4ng/ml, wherein the SB 431542 is at a concentration of between 1 to 30 μ M, or at about 10 μ M, and wherein the VEGF₁₆₅ is at a concentration of between 10 to 100 ng/ml, or at about 50ng/ml.

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14. The method of claim 13, wherein the FGF2 is at a concentration of 4ng/ml, wherein the SB 431542 is at a concentration of 10 μ M, and wherein the VEGF₁₆₅ is at a concentration of 50ng/ml.

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15. The method of any one of claims 3 to 14, wherein the first culture medium does not comprise an activator of the Wnt signaling pathway.

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16. The method of any one of the preceding claims, wherein the endothelial lineage cells to be cultured on the extracellular matrix in (b) are characterized by the expression of one or more markers, wherein the one or more markers comprise at least one marker selected from the group consisting of PECAM-1, CD144 (VE-cadherin), Von Willebrand factor (vWF) and Endothelial NOS (eNOS) and combinations thereof.

17. The method of claim 16, wherein the endothelial lineage cells to be cultured on the extracellular matrix in (b) are characterized by the expression of PECAM-1, CD144 (VE-cadherin), Von Willebrand factor (vWF) and Endothelial NOS (eNOS).

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18. The method of any one of the preceding claims, wherein the lateral plate mesoderm cells to be cultured in the extracellular matrix in (b) are further characterized by the uptake of fluorescently-labelled acetylated low-density lipoprotein.

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