CLAIMS

1. A method of culturing cells, comprising:

(a) providing a microfluidic device comprising a first chamber, a second chamber and a membrane located at an interface region between the first chamber and the second chambers to separate the first chamber from the second chamber, the membrane comprising a first surface and a second surface, and a first extracellular matrix gel comprising Collagen I and stellate cells on the first surface;

(b) seeding viable hepatocytes on the first surface, in which the gel is between the hepatocytes and the first surface of the membrane, and seeding viable endothelial cells on the second surface;

(c) overlaying the hepatocytes with a second extracellular matrix;

(d) perfusing at least one of the first chamber and the second chamber with at least one fluid that comprises a test compound, or introducing a second fluid comprising the test compound, such that the hepatocytes, the endothelial cells or both come in contact with the test compound;

(e) culturing the seeded cells under flow conditions such that the cells remain viable for at least 28 days; and

(f) detecting a higher level of activation of the stellate cells in the gel in the presence of the test compound compared to a level of activation of the stellate cells that are not exposed to the test compound, thereby determining that the test compound is toxic to the hepatocytes.

2. A method according to Claim 1, in which the hepatocytes are primary hepatocytes.

3. A method of culturing cells, comprising:

(a) providing:

(i) a microfluidic device comprising a first chamber, a second chamber, a removable top, and a membrane, the membrane located at an interface region between the first chamber and the second chamber to separate the first chamber from the second chamber, and the membrane comprising a first surface and a second surface;

(ii) hepatocytes, endothelial cells and stellate cells;

(iii) material capable of forming a gel; and

(iv) an extracellular matrix composition;

(b) removing the removable top and introducing the material into the microfluidic device such that a gel forms in the first chamber, in the second chamber, or in both chambers;

(c) introducing in any order:

(i) the hepatocytes into the first chamber such that the hepatocytes either affix to the first surface of the membrane or the gel is between the hepatocytes and the first surface of the membrane;

(ii) the endothelial cells such that the endothelial cells either affix to the second surface of the membrane or the gel is between the endothelial cells and the second surface of the membrane; and

(iii) the stellate cells embedded in the gel;

(d) overlaying the hepatocytes with the extracellular matrix composition;

(e) perfusing at least one of the first chamber and the second chamber with at least one fluid that comprises a test compound such that the hepatocytes, the endothelial cells or both come in contact with the test compound;

(f) culturing the cells under flow conditions with media; and

(g) detecting a higher level of activation of the stellate cells in the gel in the presence of the test compound compared to a level of activation of the stellate cells that are not exposed to the test compound, thereby determining that the test compound is toxic to the hepatocytes.

4. A method according to Claim 3, further comprising (h) adding additional cell types over, under or within the gel, the additional cell type of Kupffer cells.

5. A method according to Claim 3, in which the hepatocytes are selected from the group consisting of viable rat hepatocytes, viable dog hepatocytes and viable human hepatocytes.

6. A method of culturing cells, comprising:

(a) providing:

(i) a microfluidic device comprising a first chamber, a second chamber, a gel comprising Collagen I, a membrane, the membrane located at an interface region between the first chamber and the second chamber to separate the first chamber from the second chamber, and the membrane comprising a first surface and a second surface; and

(ii) hepatocytes, endothelial cells and Stellate cells;

(b) introducing in any order:

(i) the hepatocytes into the first chamber such that the hepatocytes either affix to the first surface of the membrane or the gel is between the hepatocytes and the first surface of the membrane;

(ii) the endothelial cells such that the endothelial cells either affix to the second surface of the membrane or the gel is between the endothelial cells and the second surface of the membrane; and

(iii) Stellate cells within the gel, in which the gel inhibits activation and/or migration of the Stellate cells; and

(c) perfusing at least one of the first chamber and the second chamber with at least one fluid that comprises a test compound such that the hepatocytes, the endothelial cells or both come in contact with the test compound;

(d) culturing the cells under flow conditions with media; and

(e) detecting a higher level of activation of the stellate cells within the gel in the presence of the test compound compared to a level of activation of the stellate cells that are not exposed to the test compound, thereby determining that the test compound is toxic to the hepatocytes.

7. A method for culturing cells, comprising:

(a) providing a fluidic device comprising a first structure defining a first chamber, a second structure defining a second chamber, and a membrane located at an interface region between the first chamber and the second chamber, the membrane including a first side facing toward the first chamber and a second side facing toward the second chamber;

(b) introducing a first extracellular matrix gel comprising Collagen I and stellate cells into the first chamber on the first side of the membrane;

(c) seeding hepatocytes in the first chamber on the first extracellular matrix gel and endothelial cells in the second chamber on the second side of the membrane;

(d) overlaying the hepatocytes with a second extracellular matrix;

(e) perfusing at least one of the first chamber and second chamber with at least one fluid;

(f) disposing a test compound into the fluid, or introducing a second fluid comprising the test compound, such that hepatocytes, endothelial cells or both come in contact with the test compound;

(g) stopping the perfusing in the first chamber, the second chamber, or both, so as to increase the residence time of the test compound within the fluidic device; and

(h) detecting a higher level of activation of the stellate cells in the gel in the presence of the test compound compared to a level of activation of the stellate cells that are not exposed to the test compound, thereby determining that the test compound is toxic to the hepatocytes.

8. A method according to Claim 7, further comprising (i) assessing the clearance of the test compound.

9. A method according to Claim 8, in which the assessing the clearance comprises measuring the disappearance of the test compound.

10. A method for culturing cells, comprising:

(a) providing a fluidic device comprising a first structure defining a first chamber, a second structure defining a second chamber, and a membrane located at an interface region between the first chamber and the second chamber, the membrane including a first side facing toward the first chamber and a second side facing toward the second chamber;

(b) introducing a first extracellular matrix gel comprising Collagen I and stellate cells into the first chamber on the first side of the membrane;

(c) seeding hepatocytes in the first chamber on the first extracellular matrix gel and endothelial cells in the second chamber on the second side of the membrane;

(d) overlaying the hepatocytes with a second extracellular matrix;

(e) perfusing at least one of the first chamber or second chamber with at least one fluid;

(f) disposing a test compound into the fluid such that the hepatocytes, endothelial cells or both come in contact with the test compound;

(g) recirculating the fluid so as to increase the interaction time of the test compound with the cells; and

(h) detecting a higher level of activation of the stellate cells in the gel in the presence of the test compound compared to a level of activation of the stellate cells that are not exposed to the test compound, thereby determining that the test compound is toxic to the hepatocytes.

11. A method according to Claim 10, further comprising (i) assessing the clearance of the test compound.

CLAIMS

1. A method of culturing cells, comprising:

(a) providing a microfluidic device comprising a first chamber, a second chamber and a membrane located at an interface region between the first chamber and the second chambers to separate the first chamber from the second chamber, the membrane comprising a first surface and a second surface, and a first extracellular matrix gel comprising Collagen I and stellate cells on the first surface;

(b) seeding viable hepatocytes on the first surface, in which the gel is between the hepatocytes and the first surface of the membrane, and seeding viable endothelial cells on the second surface;

(c) overlaying the hepatocytes with a second extracellular matrix;

(d) perfusing at least one of the first chamber and the second chamber with at least one fluid that comprises a test compound, or introducing a second fluid comprising the test compound, such that the hepatocytes, the endothelial cells or both come in contact with the test compound;

(e) culturing the seeded cells under flow conditions such that the cells remain viable for at least 28 days

in which the stellate cells, in response to test compounds that show toxic effects on liver cells, have a higher level of migration in the extracellular matrix gel as compared to the level of migration of stellate cells in microfluidic chips lacking the extracellular matrix gel.

2. A method according to Claim 1, in which the hepatocytes are primary hepatocytes.

3. A method of culturing cells, comprising:

(a) providing:

(i) a microfluidic device comprising a first chamber, a second chamber, a removable top, and a membrane, the membrane located at an interface region between the first chamber and the second chamber to separate the first chamber from the second chamber, and the membrane comprising a first surface and a second surface;

(ii) hepatocytes, endothelial cells and stellate cells;

(iii) material capable of forming a gel; and

(iv) an extracellular matrix composition;

(b) removing the removable top and introducing the material into the microfluidic device such that a gel forms in the first chamber, in the second chamber, or in both chambers;

(c) introducing in any order:

(i) the hepatocytes into the first chamber such that the hepatocytes either affix to the first surface of the membrane or the gel is between the hepatocytes and the first surface of the membrane;

(ii) the endothelial cells such that the endothelial cells either affix to the second surface of the membrane or the gel is between the endothelial cells and the second surface of the membrane; and

(iii) the stellate cells embedded in the gel;

(d) overlaying the hepatocytes with the extracellular matrix composition;

(e) perfusing at least one of the first chamber and the second chamber with at least one fluid that comprises a test compound such that the hepatocytes, the endothelial cells or both come in contact with the test compound;

(f) culturing the cells under flow conditions with media; and

wherein the stellate cells, in response to test compounds that show toxic effects on liver cells, have a higher level of migration in the extracellular matrix gel as compared to the level of migration of stellate cells in microfluidic chips lacking the extracellular matrix gel.

4. A method according to Claim 3, further comprising (h) adding additional cell types over, under or within the gel, the additional cell types comprising Kupffer cells.

5. A method according to Claim 3, in which the hepatocytes are selected from the group consisting of viable rat hepatocytes, viable dog hepatocytes and viable human hepatocytes.

6. A method of culturing cells, comprising:

(a) providing:

(i) a microfluidic device comprising a first chamber, a second chamber, a gel comprising Collagen I, a membrane, the membrane located at an interface region between the first chamber and the second chamber to separate the first chamber from the second chamber, and the membrane comprising a first surface and a second surface; and

(ii) hepatocytes, endothelial cells and Stellate cells;

(b) introducing in any order:

(i) the hepatocytes into the first chamber such that the hepatocytes either affix to the first surface of the membrane or the gel is between the hepatocytes and the first surface of the membrane;

(ii) the endothelial cells such that the endothelial cells either affix to the second surface of the membrane or the gel is between the endothelial cells and the second surface of the membrane; and

(iii) Stellate cells within the gel, in which the gel inhibits activation and/or migration of the Stellate cells;

(c) perfusing at least one of the first chamber and the second chamber with at least one fluid that comprises a test compound such that the hepatocytes, the endothelial cells or both come in contact with the test compound;

(d) culturing the cells under flow conditions with media;

in which the stellate cells, in response to test compounds that show toxic effects on liver cells, have a higher level of migration in the extracellular matrix gel as compared to the level of migration of stellate cells in microfluidic chips lacking the extracellular matrix gel.

7. A method for culturing cells, comprising:

(a) providing a fluidic device comprising a first structure defining a first chamber, a second structure defining a second chamber, and a membrane located at an interface region between the first chamber and the second chamber, the membrane including a first side facing toward the first chamber and a second side facing toward the second chamber;

(b) introducing a first extracellular matrix gel comprising Collagen I and stellate cells into the first chamber on the first side of the membrane;

(c) seeding hepatocytes in the first chamber on the first extracellular matrix gel and endothelial cells in the second chamber on the second side of the membrane;

(d) overlaying the hepatocytes with a second extracellular matrix;

(e) perfusing at least one of the first chamber and second chamber with at least one fluid;

(f) disposing a test compound into the fluid, or introducing a second fluid comprising the test compound, such that hepatocytes, endothelial cells or both come in contact with the test compound;

(g) stopping the perfusing in the first chamber, the second chamber, or both, so as to increase the residence time of the test compound within the fluidic device; in which the stellate cells, in response to test compounds that show toxic effects on liver cells, have a higher level of migration in the extracellular matrix gel as compared to the level of migration of stellate cells in microfluidic chips lacking the extracellular matrix gel.

8. A method according to Claim 7, further comprising (i) assessing the clearance of the test compound.

9. A method according to Claim 8, in which the assessing the clearance comprises measuring the disappearance of the test compound.

10. A method for culturing cells, comprising:

(a) providing a fluidic device comprising a first structure defining a first chamber, a second structure defining a second chamber, and a membrane located at an interface region between the first chamber and the second chamber, the membrane including a first side facing toward the first chamber and a second side facing toward the second chamber;

(b) introducing a first extracellular matrix gel comprising Collagen I and stellate cells into the first chamber on the first side of the membrane;

(c) seeding hepatocytes in the first chamber on the first extracellular matrix gel and endothelial cells in the second chamber on the second side of the membrane;

(d) overlaying the hepatocytes with a second extracellular matrix;

(e) perfusing at least one of the first chamber or second chamber with at least one fluid;

(f) disposing a test compound into the fluid such that the hepatocytes, endothelial cells or both come in contact with the test compound;

(g) recirculating the fluid so as to increase the interaction time of the test compound with the cells; in which the stellate cells, in response to test compounds that show toxic effects on liver cells, have a higher level of migration in the extracellular matrix gel as compared to the level of migration of stellate cells in microfluidic chips lacking the extracellular matrix gel.

11. A method according to Claim 10, further comprising (i) assessing the clearance of the test compound.