SUMMARY

[0005a] In one aspect, the present disclosure refers to a method of deriving hepatic macrophages from pluripotent stem cells, comprising: (a) culturing pluripotent stem cells to obtain monocytes; and (b) culturing the monocytes from (a) in a hepatic macrophage culture medium, wherein the hepatic macrophage culture medium comprises a conditioned medium and a basal medium, wherein the conditioned medium is obtained by a method comprising: (i) culturing hepatocytes in a serum-free cell culture medium in the presence of an extracellular matrix for 1 to 7 days; and (ii) isolating the supernatant at the end of the culturing process in (i). In another aspect, the present disclosure refers to a method of deriving hepatic macrophages from induced pluripotent stem cells, comprising: (a) culturing the induced pluripotent stem cells to obtain monocytes; and (b) culturing the monocytes from (a) in a hepatic macrophage culture medium in the absence of an extracellular matrix, wherein the hepatic macrophage culture medium comprises a conditioned medium and a basal medium, and wherein the conditioned medium is obtained by a method comprising: (i) culturing hepatocytes in a serum-free cell culture medium in the presence of an extracellular matrix for 1 to 7 days; and (ii) isolating the supernatant at the end of the culturing process in (i). In yet another aspect, the present disclosure refers to a kit for deriving hepatic macrophages from monocytes, comprising a cell culture device not coated using extracellular matrix, a conditioned medium and a basal medium, wherein the conditioned medium is obtained by a method comprising: (i) culturing hepatocytes in a serum-free cell culture medium in the presence of an extracellular matrix for 1 to 7 days and (ii) isolating the supernatant at the end of the culturing process in (i).

[0006] In one example, there is provided a method of deriving hepatic macrophages from monocytes, comprising culturing the monocytes in a hepatic macrophage culture medium, wherein the hepatic macrophage culture medium comprises a conditioned medium and a basal medium, wherein the conditioned medium is obtained by a method comprising: (a) culturing hepatocytes in a serum-free cell culture medium in the presence of an extracellular matrix for 1 to 7 days; and (b) isolating the supernatant at the end of the culturing process in (a).

[0007] In another example, there is provided a method of deriving hepatic macrophages from pluripotent stem cells, comprising: (a) culturing pluripotent stem cells to obtain monocytes; and (b) culturing the monocytes from (a) in a hepatic macrophage culture medium, wherein the hepatic macrophage culture medium comprises a conditioned medium and a basal medium, wherein the conditioned medium is obtained by a method comprising: (i) culturing hepatocytes in a serum-free cell culture medium in the presence of an extracellular

matrix for 1 to 7 days; and (ii) isolating the supernatant at the end of the culturing process in (i). In one example, (a) culturing pluripotent stem cells to obtain monocytes further comprises: (i) culturing pluripotent stem cells to obtain embryoid bodies; and (ii) differentiating embryoid bodies to obtain monocytes. In one example, the pluripotent stem cells are induced pluripotent stem cells. In one example, culturing the monocytes in a hepatic macrophage culture medium in the method of the first aspect and/or the second aspect comprises culturing the monocytes in the hepatic macrophage culture medium in the absence of an extracellular matrix.

[0008] In one example, the basal medium in the the hepatic macrophage culture medium is a low serum medium. In one example, the ratio of the conditioned medium and the basal medium in the hepatic macrophage culture medium is in the range of 10:1 to 1:10, or 1:1. In another example, the serum level in the low serum basal medium is at a percentage of 0.1% to 10%.

[0009] Also disclosed herein is a kit for deriving hepatic macrophages from monocytes, comprising a conditioned medium and a basal medium, wherein the conditioned medium is obtained by a method comprising: (i) culturing hepatocytes in a serum-free cell culture medium in the presence of an extracellular matrix for 1 to 7 days; and (ii) isolating the supernatant at the end of the culturing process in (i).

[0010] In one example, the kit of the third aspect further comprises a cell culture device that is not coated using extracellular matrix. In another example, the basal medium in the kit is adapted to be used as a low serum basal medium. In yet another example, the kit further comprises one or more of the following supplements for the basal medium: insulin, transferrin, selenous acid, albumin, fatty acids, glutamine supplements and buffering agent.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] 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:

[0012] **Figure 1** shows schematics for generation of hPSC-hepatic-macrophages (hPSC-KCs) from human pluripotent stem cells (hPSCs). hPSC-Monocytes (hPSC-Mon) were derived from hPSCs and differentiated into hPSC-KCs or hPSC-non-hepatic-macrophages (NL-Mφ), depending on the culturing conditions. **Figure 1A** shows formation and attachment of embryoid bodies (EB) and generation of non-adherent hPSC-Mon which can be harvested from the culture supernatant. hPSC-Mon were treated for additional seven days in optimized primary human hepatocyte conditioned medium to generate hPSC-KCs. Scale bar: 200 μm.

CLAIMS

1. A method of deriving hepatic macrophages from pluripotent stem cells, comprising:

(a) culturing pluripotent stem cells to obtain monocytes; and

(b) culturing the monocytes from (a) in a hepatic macrophage culture medium, wherein the hepatic macrophage culture medium comprises a conditioned medium and a basal medium, wherein the conditioned medium is obtained by a method comprising:

(i) culturing hepatocytes in a serum-free cell culture medium in the presence of an extracellular matrix for 1 to 7 days; and

(ii) isolating the supernatant at the end of the culturing process in (i).

2. The method of claim 1, wherein culturing pluripotent stem cells further comprises:

(i) culturing pluripotent stem cells to obtain embryoid bodies; and

(ii) differentiating embryoid bodies to obtain monocytes.

3. The method of claim 2 or 3, wherein the pluripotent stem cells are induced pluripotent stem cells.

4. The method of any of the preceding claims, wherein the basal medium is a low serum basal medium.

5. The method of any of the preceding claims, wherein culturing the monocytes in a hepatic macrophage culture medium comprises culturing the monocytes in the hepatic macrophage culture medium in the absence of an extracellular matrix.

6. The method of any of the preceding claims, wherein the ratio of the conditioned medium and the basal medium is in the range of 10:1 to 1:10.

7. The method of any of the preceding claims, wherein the ratio of the conditioned medium and the basal medium is 1:1.

8. The method of any one of claims 4 to 7, wherein the serum level in the low serum basal medium is at a percentage of 0.1% to 10%.

9. A method of deriving hepatic macrophages from induced pluripotent stem cells, comprising:
(a) culturing the induced pluripotent stem cells to obtain monocytes; and
(b) culturing the monocytes from (a) in a hepatic macrophage culture medium in the absence of an extracellular matrix, wherein the hepatic macrophage culture medium comprises a conditioned medium and a basal medium, and wherein the conditioned medium is obtained by a method comprising:
(i) culturing hepatocytes in a serum-free cell culture medium in the presence of an extracellular matrix for 1 to 7 days; and
(ii) isolating the supernatant at the end of the culturing process in (i).

10. A kit for deriving hepatic macrophages from monocytes, comprising a cell culture device not coated using extracellular matrix, a conditioned medium and a basal medium, wherein the conditioned medium is obtained by a method comprising:
(i) culturing hepatocytes in a serum-free cell culture medium in the presence of an extracellular matrix for 1 to 7 days and

(ii) isolating the supernatant at the end of the culturing process in (i).

11. The kit of claim 10 , wherein the basal medium is adapted to be used as a low serum basal medium.

12. The kit of any one of claims 10 to 11, further comprising one or more of the following supplements for the basal medium: insulin, transferrin, selenous acid, albumin, fatty acids, glutamine supplements and buffering agent.