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SUMMARY

Methods are provided for producing a population of hepatocyte-like cells (iHeps) from a population of adipocyte-derived stem cells (ASCs). The methods include placing a population of ASCs into a three dimensional culture (e.g., hanging drop suspension culture, high density culture, spinner flask culture, microcarrier culture, etc.) and contacting the cells with a first and second culture medium. Also provided are methods of treating an individual, which include producing a population of iHeps from a population of ASCs, and administering an effective number of iHeps into the individual.

The presence of iHeps may be confirmed by various methods, including for example, contacting the induced cell population with an antibody specific for a hepatocyte marker protein, and determining the percentage of cells positive for expression. In some embodiments, 15% or more of cells of the induced cell population are hepatocyte-like cells. In some cases, 37% or more of the cells of the induced cell population are hepatocyte-like cells. In some embodiments, the elapsed time to generate iHeps from ASCs is less than 13 days (e.g., less than 10 days). The high efficiency of iHep production and the short time frame over which iHeps can be produced by the subject methods facilitate treatment methods because liver damage (e.g., liver failure, e.g., due to acetaminophen toxicity) can rapidly cause death (e.g., in 2 weeks or less). Kits for practicing the methods of the disclosure are also provided.

In one aspect of the present disclosure, there is provided a method of producing a population of hepatocyte-like cells from a population of adipose~~cyte~~-derived stem cells (ASCs), the method comprising:(a) placing a population of ASCs into a three dimensional culture selected from the group consisting of: stirred suspension culture, hanging drop suspension culture, high density cell culture, spinner flask culture, microcarrier culture, and bioreactor culture, for 3 days or less~~a period of time sufficient~~ to produce an ASC-derived cellular aggregate;

(b) contacting cells of the ASC-derived cellular aggregate with a first culture medium comprising Activin A at a concentration in a range of from 50 ng/mL to 150 ng/mL and fibroblast growth factor (FGF) at a concentration in a range of from 5 ng/mL to 50 ng/mL, for 7 days or less, to produce a precursor cell population; and

(c) contacting cells of the precursor cell population with a second culture medium comprising hepatocyte growth factor (HGF) at a concentration in a range of from 75 ng/mL to 250 ng/mL and an FGF at a concentration in a range of from 5 ng/mL to 50 ng/mL to produce an induced cell population that comprises hepatocyte-like cells,

wherein the total time elapsed from beginning step (a) to the production of the induced cell population in step (c) is less than 13 days.

In another aspect of the present disclosure, there is provided a use of a composition comprising hepatocyte-like cells produced by the method disclosed herein, wherein 25% or more of the cells of the cell population are hepatocyte-like cells, in the manufacture of a medicament for treating an individual with liver disease and/or liver damage, wherein an effective number of the hepatocyte-like cells is to be administered to the individual to improve liver function.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is best understood from the following detailed description when read in conjunction with the accompanying drawings. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures.

Figures 1A-E provide a comparison of SCi-Heps to Chi-Heps, and a comparison of the methods of generating SCi-Heps and Chi-Heps. (**A**) The two different methods for

Claims:

1. A method of producing a population of hepatocyte-like cells from a population of ~~adipocyte~~adipose-derived stem cells (ASCs), the method comprising:

(a) placing a population of ASCs into a three dimensional culture selected from the group consisting of: stirred suspension culture, hanging drop suspension culture, high density cell culture, spinner flask culture, microcarrier culture, and bioreactor culture, for ~~a period of time sufficient~~3 days or less to produce an ASC-derived cellular aggregate;

(b) contacting cells of the ASC-derived cellular aggregate with a first culture medium comprising Activin A at a concentration in a range of from 50 ng/mL to 150 ng/mL and a fibroblast growth factor (FGF) at a concentration in a range of from 5 ng/mL to 50 ng/mL, for 7 days or less, to produce a precursor cell population; and

(c) contacting cells of the precursor cell population with a second culture medium comprising hepatocyte growth factor (HGF) at a concentration in a range of from 75 ng/mL to 250 ng/mL and an FGF at a concentration in a range of from 5 ng/mL to 50 ng/mL to produce an induced cell population that comprises hepatocyte-like cells,

wherein the total time elapsed from beginning step (a) to the production of the induced cell population in step (c) is less than 13 days.

2. The method of claim 1, wherein the three dimensional culture is a high density cell culture selected from a spinner flask culture and a bioreactor culture.

3. The method of claim 1 or claim 2, wherein the three dimensional culture is a microcarrier culture.

4. The method of claim 1, wherein the three dimensional culture is a hanging drop suspension culture.

5. The method of any of claims 1-4, wherein:

~~(i) the population of ASCs of step (a) is placed into the three dimensional culture for 3 days or less to produce the ASC derived cellular aggregate; and/or~~

(~~i~~ii) the contacting of step (b) is for 3 days or less; and/or

(~~i~~iii) the contacting of step (c) is for 8 days or less; and/or

(i~~v~~vii) the total time elapsed from beginning step (a) to the production of an induced cell population in step (c) is ~~less than 13~~10 days or less.

6. The method of any of claims 1-5, wherein the FGF is FGF4.

7. The method of any of claims 1-6, wherein the first culture medium further comprises a Wnt signaling agonist.

8. The method of any of claims 1-7, wherein the second culture medium further comprises a compound selected from the group consisting of: oncostatin M (OSM), dexamethasone (Dex), Dimethyl sulfoxide (DMSO), and a combination thereof.

9. The method of any of claims 1-8, further comprising determining the percentage of cells of the induced cell population that are hepatocyte-like cells.

10. The method of any of claims 1-9, wherein 11% or more of the cells of the induced cell population hepatocyte-like cells.

11. The method of any of claims 1-10, further comprising enriching the induced cell population for hepatocyte-like cells.

12. The method of any of claims 1-11, further comprising preserving the hepatocyte-like cells for future use.

13. Use of a composition comprising hepatocyte-like cells produced by the method of any of claims 1-12, wherein 25% or more of the cells of the cell population are hepatocyte-like cells, in the manufacture of a medicament for treating an individual with liver disease and/or liver damage, wherein an effective number of the hepatocyte-like cells is to be administered to the individual to improve liver function.

14. The use according to claim 13, wherein the hepatocyte-like cells of the composition are preserved for future use.

15. The use according to claim 13 or claim 14, wherein at least $1x10^4$ hepatocyte-like cells are to be administered.

16. The use according to any of claims 13-15, wherein the hepatocyte-like cells are to be transplanted into the individual's liver.

17. The use according to any of claims 13-16, wherein the ASCs used in the method to produce the hepatocyte-like cells are autologous to the individual being treated.

18. The use according to any of claims 13-16, wherein the ASCs used in the method to produce the hepatocyte-like cells are not from the individual being treated and are instead from a related or unrelated individual.