Epicardial cells from infarcted animals primed with Tβ4 *in vivo* differentiate to cells that express cardiomyocyte markers in explant cultures[15].

[0008] Although these advances have enabled the efficient and scalable derivation of cardiomyocytes from hPSCs, these differentiated populations are not optimal for many applications, as they contain immature cells and the proportion of different cardiac lineage cells including myocardial and epicardial within them is not well defined. To realize the potential of hPSCs in cardiovascular research and therapeutic applications, it will likely be necessary to develop culture systems and engineered tissues that more accurately represent the human heart–.

Summary

[0008a] In one aspect, there is provided a method of specifying a WT1+ cardiovascular progenitor cell population comprising the step of contacting an hPSC-derived KDR+ and PDGFRalpha+ cardiovascular mesoderm cell population with a cardiovascular progenitor specification cocktail comprising human BMP4 at a concentration of between 0.63 ng/ml and 10 ng/ml.

[0008b] In another aspect, there is provided a method of producing an epicardial lineage cell population comprising the step of contacting a WT1+ cardiovascular progenitor cell population with a maturation cocktail comprising suitable cell culture components in a suitable cell culture format for a period of from 2 to 20 days.

[0008c] In another aspect, there is provided a method of preparing an epicardial lineage cell population and causing the population to further differentiate comprising the steps of: (a) specifying a WT1+ cardiovascular progenitor cell population by a method comprising contacting an hPSC-derived KDR+ and PDGFRalpha+ cardiovascular mesoderm cell population with a cardiovascular progenitor specification cocktail comprising human BMP4 at a concentration of between 0.63 ng/ml and 10 ng/ml; (b) contacting the WT1+ cardiovascular progenitor cell population with a maturation cocktail comprising suitable cell culture components in a suitable cell culture format for a period of from 2-20 days; (c) passaging the cell population, then culturing the cell population in a larger cell culture format in a suitable medium for one day, optionally followed by one of the following regimens comprising: (i) causing the cell population to progress toward a vascular smooth muscle-like fate by treating it with human TGFβ-1 for a period of time; (ii) causing the cell population to progress toward a vascular smooth muscle-like

fate by treating it with human TGFβ-1 for a period of time, followed by treating it with human bFGF for a period of time; (iii) causing the cell population to progress toward a fibroblast-like fate by treating it with human bFGF.

[0009] An ~~aspect~~ example includes a method of obtaining a cardiovascular lineage cell population, optionally cardiomyocyte lineage cell population or an epicardial lineage cell population from human pluripotent stem cells (hPSCs) comprising the steps: (a) contacting BMP component primed hPSCs with a cardiovascular mesoderm programming cocktail suitable for inducing the hPSCs to differentiate to a cardiovascular mesoderm cell population under conditions suitable for the programming cocktail to penetrate the hPSCs and culturing the contacted hPSCs for a period of time to generate a KDR+ and PDGFRα+ cardiovascular mesoderm cell population; (b) contacting the cardiovascular mesoderm cell population with a cardiovascular progenitor specification cocktail suitable to specify a NKX2-5+ or WT1+ cardiovascular progenitor cell population under conditions suitable for the specification cocktail to penetrate the cardiovascular mesoderm cell population and culturing the contacted cardiovascular mesoderm cell population for a period of time to generate a NKX2-5+ or WT1+ cardiovascular progenitor cell population; and (d) contacting the cardiovascular progenitor cell population with a maturation cocktail under conditions suitable for the maturation cocktail to penetrate the cardiovascular progenitor cell population and culturing the contacted cardiovascular progenitor population for a period of time to produce a cardiovascular lineage population optionally a cardiomyocyte lineage cell population expressing cardiac troponin T (cTnT) and/or SIRPA and/or an epicardial lineage cell population optionally expressing WT1 and/or comprising epicardial derived cells (EPDCs).

[0010] Other features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred ~~embodiment~~examples of the disclosure are given by way of illustration only, since various changes and modifications within the spirit

Claims:

~~1. A method of obtaining a cardiomyocyte lineage or an epicardial lineage cell population from human pluripotent stem cells (hPSCs) comprising the steps: (a) contacting BMP component primed hPSCs with a cardiovascular mesoderm programming cocktail suitable for inducing the hPSCs to differentiate to a cardiovascular mesoderm cell population under conditions suitable for the programming cocktail to penetrate the hPSCs and culturing the contacted hPSCs for a period of time to generate a KDR+ and PDGFRalpha+ cardiovascular mesoderm cell population; (b) contacting the cardiovascular mesoderm cell population with a cardiovascular progenitor specification cocktail suitable to specify a NKX2-5+ or WT1+ cardiovascular progenitor cell population under conditions suitable for the specification cocktail to penetrate the cardiovascular mesoderm cell population and culturing the contacted cardiovascular mesoderm cell population for a period of time to generate a NKX2-5+ or WT1+ cardiovascular progenitor cell population; and (d) contacting the cardiovascular progenitor cell population with a maturation cocktail under conditions suitable for the maturation cocktail to penetrate the cardiovascular progenitor cell population and culturing the contacted cardiovascular progenitor population for a period of time to produce a cardiovascular population, optionally cardiomyocyte lineage cells expressing cardiac troponin T (cTnT) and/or SIRPA and/or epicardial lineage cells expressing WT1.~~

~~2. The method of claim 1 wherein the BMP component primed hPSCs are prepared by contacting the hPSCs with BMP component for about 1 to about 2 days, optionally wherein the BMP component is BMP4 and/or BMP2.~~

~~3. The method of claims 1 or 2, wherein the cardiovascular mesoderm programming cocktail comprises a BMP component and an activin component and optionally a FGF component and the cardiovascular mesoderm programming cocktail is contacted with the hPSCs for about 3 to about 5 days.~~

1. A method of specifying a WT1+ cardiovascular progenitor cell population comprising the step of contacting an hPSC-derived KDR+ and PDGFRalpha+ cardiovascular mesoderm cell population with a cardiovascular progenitor specification cocktail comprising human BMP4 at a concentration of between 0.63 ng/ml and 10 ng/ml.

2. The method of claim 1, wherein the cardiovascular progenitor specification cocktail further comprises CHIR99021.

3. The method of claim 2, wherein CHIR99021 is present at a concentration of between 1 µM and 4 µM.

4. The method of claim 3, wherein the ~~FGF component is bFGF~~cardiovascular progenitor specification cocktail further comprises SB431542.

~~5. The method of claim 3 wherein the BMP component is BMP4.~~

~~6.~~ 5. The method of claim ~~3~~4, wherein the ~~activin component is Activin A.~~

~~7. The method of any one of claims 1 to 6, wherein the hPSCs are comprised in embryoid bodies.~~

~~8. A method for obtaining a NKX2-5+ or WT1+~~ cardiovascular progenitor ~~cell population from hPSCs comprising the steps: (a) obtaining a KDR+ and PDGFRalpha+ cardiovascular mesoderm cell population from hPSCs optionally as defined in step a of claim 1; (b) contacting the KDR+ and PDGFRalpha+ cardiovascular mesoderm cell population with a cardiovascular progenitor specification cocktail under conditions suitable for the~~ specification cocktail ~~to penetrate the cardiovascular mesoderm cell population and culturing the contacted cardiovascular mesoderm cell population for a period of time sufficient to generate a NKX2-5+ or WT1+ cardiovascular progenitor cell population~~further comprises human VEGF.

~~9~~6. The method of ~~any one of claims 1 to 8~~claim 5, wherein the ~~KDR+~~human VEGF is present at a concentration of 5 ng/ml and ~~PDGFRalpha+ cardiovascular mesoderm cell population is dissociated prior to contacting with~~ the ~~cardiovascular progenitor specification cocktail~~SB431542 is present at a concentration of 5.4 µM.

~~10~~7. The method of ~~any one of claims 1 to 9~~claim 3, wherein the cardiovascular mesoderm cell population is contacted with the cardiovascular progenitor specification cocktail for at least 12 hours to about 48 hours.

~~11.~~8. The method of ~~any one of claims~~claim 1 ~~to 10~~, wherein the hPSC-derived KDR+ and PDGFRalpha+ cardiovascular mesoderm cell population is dissociated prior to the step of contacting with the cardiovascular progenitor specification cocktail ~~comprises a cardiomyocyte lineage promoting component, wherein the cardiomyocyte promoting component is in a suitable concentration for promoting cardiomyocyte development and specifies a NKX2-5+ cardiovascular progenitor population~~.

~~12. The method of claim 11, wherein the cardiomyocyte promoting component is noggin at a concentration of less than 200 ng/mL, less than 150 ng/mL, less than 100 ng/mL, less than 50 ng/mL, or less than 25 ng/mL and/or greater than 12.5 ng/mL.~~

~~13.The method of claim 11, wherein the cardiomyoctye promoting component is dorsomorphin less than 1 µM, less than 0.5 µM, less than 0.25 µM, or less than 0.1 µM.~~

9. ~~14.~~The method of claim ~~11, wherein the cardiomyocyte promoting component is BMP4 at a concentration of less than 0.63, less than 0.5 ng/mL, less than 0.4 ng/mL, or less than 0.3 ng/mL.~~

~~15. The method of claim 11~~1, wherein the cardiovascular ~~progenitor specification cocktail comprises: 1) a combination of a Wnt inhibitor optionally selected from, DKK1, XAV939 and IWP2 and a BMP component, optionally wherein the BMP component is BMP4 at a concentration of at least 0.01 ng/mL, at least 0.05 ng/mL, at least 0.1 ng/mL, at least 0.5 ng/mL, at least 1.25 ng/mL, at least 2.5 ng/mL, at least 5 ng/mL, but less than 10ng/ml, or less than 15 ng/mL or preferably about 0.5 ng/mL; or 2) a BMP inhibitor, such as noggin or dorsomorphin, optionally noggin at a concentration of less than 200 ng/mL, less than 150 ng/mL, less than 100 ng/mL, less than 50 ng/mL, or less than 25 ng/mL and/or greater than 12.5 ng/mL; 3) a Wnt inhibitor, and/or **4)** a cardiomyocyte lineage concentration of a BMP component, optionally BMP4 for example wherein the BMP4 is at a concentration of less than 0.63 ng/mL, less than 0.5 ng/mL, less than 0.4 ng/mL, or less than 0.3 ng/mL~~mesoderm cell population is contacted with the cardiovascular progenitor specification cocktail for at least 12 hours to about 48 hours.

~~16~~10. The method of ~~any one of claims 1 to 15~~claim 9, wherein the BMP4 concentration in the cardiovascular progenitor specification cocktail is at least 1.25 ng/ml.

11. The method of claim 10, wherein the BMP4 concentration in the ~~KDR+PDGFRα+ cardiovascular mesoderm population and/or the NKX2-5+~~ cardiovascular progenitor specification cocktail is 10 ng/ml.

12. A method of producing an epicardial lineage cell population ~~is isolated~~comprising the step of contacting a WT1+ ▪

~~17.The method of any one of claims 1 to 16, wherein the NKX2-5+~~ cardiovascular progenitor cell population ~~is further contacted~~ with a maturation cocktail comprising ~~a VEGF component.~~ suitable cell culture components in a suitable cell culture format for a period of from 2 to 20 days.

13. The method of claim 12, wherein the maturation cocktail further comprises VEGF.

14. ~~18.A method for producing cardiac troponin T+ (cTnT) cardiomyocyte lineage cell population comprising: (a) obtaining a NKX2-5+ cardiovascular progenitor population according to the method of any one of claims 1 to 16; (b) contacting the cardiovascular progenitor cell population with a maturation cocktail comprising a VEGF component under conditions suitable for the maturation cocktail to penetrate the cardiovascular progenitor cell population; and (b) culturing the contacted cardiovascular progenitor population for a period of time sufficient to produce cardiomyocytes expressing cardiac troponin T (cTnT).~~

~~19.~~The method of claim ~~18~~13, wherein the ~~NKX2-5+ cardiovascular progenitor population is contacted with the maturation cocktail for about 4 or more~~contacting takes place for a period of from 2 to 9 days~~, optionally about 4, about 5, about 9, about~~ .

15~~ or about 20~~. The method of claim 12, wherein the step of contacting takes place in a 96-well plate and, after 2-9 days, ~~optionally until mature contracting cardiomyocytes are produced.~~

~~20. A~~the cell population is passaged and cultured in a larger cell culture format than the 96-well-plate.

16. The method of claim 15, wherein the cell population is cultured in a larger cell culture format until they exhibit an epithelial morphology.

17. The method of claim 15, wherein the cell population is cultured in a larger cell culture format for 4 days.

18. ~~producing~~The method of claim 15, wherein the larger cell culture format is a 6-well plate.

19. The method of claim 14 comprising contacting the WT1+ cardiovascular progenitor cell population with the maturation cocktail for 9 days, further comprising passaging the cells, then culturing the cells in a larger format in a suitable medium for one day, followed by one of the following regimens comprising the step or steps of:

(a) causing the cells to progress toward a vascular smooth muscle-like fate by treating them with human TGFβ-1 for a period of time;

(b) causing the cells to progress toward a vascular smooth muscle-like fate by treating them with human TGFβ-1 for a period of time, followed by treating them with human bFGF for a period of time;

(c) causing the cells to progress toward a fibroblast-like fate by treating them with human bFGF.

20. The method of claim 19, wherein:

step (a) is performed and the cells are treated with human TGFβ-1 at a concentration of 5 ng/ml for 4 days, or

step (b) is performed and the cells are treated with human TGFβ-1 at a concentration of 5 ng/ml for 4 days followed by treatment of the cells with human bFGF at a concentration of 10 ng/ml for a period of 4 days, or

step (c) is performed and the cells are treated with human bFGF at a concentration of 10 ng/ml for 8 days.

21. A method of preparing an epicardial lineage cell population~~,~~ and causing the population to further differentiate comprising the steps~~:~~ of:

(a) ~~obtaining~~specifying a WT1+ cardiovascular progenitor cell population by a method comprising contacting an hPSC-derived KDR+ and PDGFRalpha+ cardiovascular mesoderm cell population ~~from hPSCs optionally as defined in step a of claim 1; (b) contacting the cardiovascular mesoderm cell population~~ with a cardiovascular progenitor specification cocktail comprising ~~an epicardial lineage promoting component under conditions~~ human BMP4 at a concentration of between 0.63 ng/ml and 10 ng/ml;

(b) contacting the WT1+ cardiovascular progenitor cell population with a maturation cocktail comprising suitable ~~for the specification cocktail to penetrate the cardiovascular mesoderm~~cell culture components in a suitable cell culture format for a period of from 2-20 days;

(c) passaging the cell population ~~and~~, then culturing the ~~contacted cardiovascular mesoderm~~ cell population ~~for~~in a ~~period of time sufficient to generate a WT1+ cardiovascular progenitor~~larger cell ~~population.~~culture format in a suitable medium for one day, optionally followed by one of the following regimens comprising:

~~21. The method of any one of claims 1 to 10 or 20, wherein the cardiovascular progenitor specification cocktail comprises an epicardial cell promoting component, optionally wherein the epicardial cell promoting component comprises BMP4 in a suitable concentration for promoting epicardial cell development.~~

~~22. The method of claim 20 or 21, wherein~~ (i) causing the ~~epicardial cell promoting component comprises BMP4 at a concentration of at least 1.25 ng/mL, at least 2.5 ng/mL, at least 5 ng/mL or at least 10 ng/mL.~~

~~23. The method of claim 21 or 22, wherein the epicardial cell promoting component further comprises a Wnt component, optionally CHIR99021.~~

24. The method of claim 20, wherein the epicardial cell promoting component comprises BMP4 and a Wnt component optionally CHIR 99021.

25. The method of any one of claims 1 to 10, 20 to 24, wherein the WT1+ cardiovascular progenitor cell population is contacted with a maturation cocktail comprising a VEGF component.

26. The method of claim 25, wherein the WT1+ cardiovascular progenitor population is contacted with the maturation cocktail for 4 or more days, optionally about 4, about 5, about 9, about 15 or about 20 days to produce a maturation cocktail contacted WT1+ epicardial lineage cell population.

27. The method of claim 26, wherein the maturation cocktail contacted WT1+ epicardial lineage cell population is isolated.

28. The method of claim 25 or 26, wherein the maturation cocktail contacted WT1+ epicardial lineage cell population is cultured to obtain a zona occludins 1 (ZO1)+ WT1+ epicardial lineage cell population, optionally wherein the ZO1+WT1+ epicardial lineage cell population is isolated.

29. The method of any one of claims 26 to 28, wherein the maturation cocktail contacted WT1+ epicardial lineage cell population and/or the ZO1+ WT1+ epicardial lineage cell population is contacted with an epithelial-to-mesenchymal transition (EMT) cocktail and cultured for a period of time.

30. The method of claim 29, wherein the EMT cocktail comprises: 1) a TGFβ component; 2) a TGFβ component and a FGF component, optionally wherein the TGFβ component and the FGF component are sequentially administered; or 3) FGF component.

31. The method of any one of claims claim wherein the EMT cocktail is contacted with the WT1+ population of cells according to the following schedule: 1) TGFβ-1 for about four days followed by about four days with no additional factor (TGFβ), 2) TGFβ-1 for about four days followed by about four days with bFGF (TGFβ+bFGF), or 3) bFGF for about eight days (bFGF).

32. The method of any one of claims 29 to 31, wherein the EMT cocktail comprises: 1) TGFβ component; or 2) a TGFβ component and a FGF component; and the cell population is cultured for a period of time to produce expression of an EMT marker such as SNAI1 or SNAI2, a mesenchymal marker such as vimentin and/or CD90 and/or a smooth muscle marker such as SMA, optionally measured by flow cytometry or expression of a smooth muscle gene optionally *CNN1, MYH11, TAGLN* and *SMTN.*

~~33. The method of any one of claims 29 to 32, wherein the population of cells is cultured until the population of cells expresses a smooth muscle marker or transcript to obtain~~to progress toward a vascular smooth muscle ~~lineage cell population, optionally until the population of cells expresses increased levels of a mesenchymal marker, optionally vimentin and/or CD90 or until a proportion of cells exhibit calcium transients upon stimulation.~~ like fate by treating it with human TGFβ-1 for a period of time;

~~34. The method of claim 29 or 30 wherein the EMT cocktail comprises: an FGF component and the~~ (ii) causing the cell population ~~is cultured~~to progress toward a vascular smooth muscle-like fate by treating it with human TGFβ-1 for a period of time, followed by treating it with human bFGF for a period of time ~~to produce~~;

(iii) causing the cell population to progress toward a fibroblast ~~lineage cell population expressing an epicardial-derived fibroblast marker optionally *TCF21*, optionally measured by qRT-PCR~~ like fate by treating it with human bFGF.

22. ~~35.~~ The method of claim ~~34~~21, wherein

(i) is performed and the ~~population of~~ cells are treated with human TGFβ-1 at a concentration of 5 ng/ml for 4 days, or

(ii) is ~~cultured to express TCF21~~performed and~~/or attain invasiveness to obtain a fibroblast lineage cell population.~~ the cells are treated with human TGFβ-1 at a concentration of 5 ng/ml for 4 days followed by treatment of the cells with human bFGF at a concentration of 10 ng/ml for a period of 4 days, or

(iii) is performed and the cells are treated with human bFGF at a concentration of 10 ng/ml for 8 days.

~~36. The method of any one of claims 1-10, and 26 to 28, wherein the maturation cocktail contacted WT1+ epicardial lineage cell population and/or the WT1+ ZO1+ epicardial lineage cell population is cultured for a period of time to obtain a retinol dehydrogenase expressing epicardial lineage cell population, optionally wherein the retinol dehydrogenase expressing epicardial lineage cell population is ALDH1A2 expressing or Aldefluor positive staining, optionally wherein the cell population is at least 50% Aldefluor™ positive.~~

~~37. The method of any one of claims 33 to 36, wherein the vascular smooth muscle lineage cell population, a fibroblast lineage cell population and/or a retinol dehydrogenase expressing epicardial lineage cell population are isolated.~~

~~38. The method of any one of claims 1 to 37, wherein cardiovascular progenitor specification cocktail further comprises an activin/nodal inhibitor, optionally SB431542.~~

39. The method of any one of claims 1 to 38, wherein the hPSCs are an induced pluripotent stem cell (iPSC) line and/or a human embryonic stem cell (hESC) line.

40. The method of claim 39, wherein the iPSC is a fibroblast derived iPSC line.

41. A population of cardiovascular lineage cells and/or cells epicardial derived cell (EPDC) produced according to the method of any one of claims 1 to 40.

42. A kit comprising: 1) an agent for measuring expression of a marker expressed on a cardiovascular lineage cell or an EPDC, the marker selected from KDR, PDGFRalpha, NKX2-5+, WT1, ZO1, EMT marker such as SNAI1 and/or SNAI2, a mesenchymal marker such as vimentin and/or CD90 and/or a smooth muscle marker such as SMA, a smooth muscle gene optionally *CNN1, MYH11, TAGLN* and *SMTN*, TCF21, retinol dehydrogenase, and/or Aldefluor activity; and/or 2) a component for inducing differentiation of a cardiovascular lineage cell population, the component selected from a BMP component in a concentration for specifying cardiomyocytes or comprising a BMP component and a Wnt component for epicardial specification.

43. A method for identifying a cardiovascular cell differentiation promotion agent comprising the steps: (a) contacting a test cell population with a test agent at a step in any one of claims 1 to 40; (b) monitoring for expression of a marker selected from KDR, PDGFRalpha, NKX2-5+, WT1, ZO1, a mesenchymal marker such as vimentin and/or CD90 and/or a smooth muscle marker such as SMA, a smooth muscle gene optionally *CNN1, MYH11, TAGLN* and *SMTN*, TCF21, retinol dehydrogenase, and/or Aldefluor activity levels in the test cell population and a control; and (c) identifying the test agent as a cardiovascular cell differentiating promotion agent when the test agent induces and/or increases expression of the cardiovascular marker.