**CLAIMS**

1. A method of generating a population of enucleated erythroid cells, the method comprising:

(a) disposing a volume of a first cell culture of erythroid progenitor cells into a second culture medium comprised within a perfusion bioreactor to provide a second cell culture with an initial cell density of about 0.1 x $10^5$ cells/mL to about 1 x $10^7$ cells/mL;

(b) perfusion culturing the second cell culture for about 2 days to about 15 days;

(c) disposing a volume of the second cell culture of step (b) into a third culture medium comprised within a perfusion bioreactor to provide a third cell culture with an initial cell density of about 1 x $10^5$ to about 1 x $10^7$ cells/mL;

(d) perfusion culturing the third cell culture of step (c) for about 5 days to 20 days,

wherein after step (d) the third culture medium comprises a population of enucleated erythroid cells.

2. The method of claim 1, wherein the method further comprises prior to step (a):

(i) disposing a plurality of erythroid progenitor cells in a first culture medium comprised within a vessel to provide the first cell culture with an initial cell density of about of about 0.1 x $10^5$ cells/mL to about 2 x $10^6$ cells/mL; and

(ii) batch or fed batch culturing the first cell culture for about 1 day to about 15 days.

3. The method of claim 2, wherein the vessel in step (i) is a shake flask, a shake tube, or a culture bag.

4. The method of claim 2 or 3, wherein step (ii) comprises batch culturing the first cell culture.

5. The method of claim 2 or 3, wherein step (ii) comprises fed batch culturing the first cell culture.

6. The method of claim 5, wherein fed batch culturing comprises adding an additional volume of the first culture medium to the first cell culture over time.

7. The method of any one of claims 2-6, wherein the first culture medium comprises one or more of Flt-3 ligand, stem cell factor (SCF), IL-3, and IL-6.

8. The method of any one of claims 2-7, wherein the first culture medium comprises:

about 1 Tg/mL to about 20 Tg/mL insulin;

about 1 mM to about 10 mM of L-glutamine, L-alanyl-L-glutamine, L-glycyl-L-glutamine, N-acetyl-L-glutamine, or a combination thereof;

lipid; and/or

about 50 Tg/mL to about 400 Tg/mL transferrin.

9. The method of any one of claims 1-8, wherein the perfusion culturing in step (b) comprises adding an additional volume of the second culture medium to the second cell culture over time.

10. The method of any one of claims 1-9, wherein the second culture medium comprises one or more of: transferrin, IL-3, SCF, dexamethasone, erythropoietin (EPO), and insulin.

11. The method of any one of claims 1-10, wherein the second culture medium comprises:

about 1 mM to about 10 mM of L-glutamine, L-alanyl-L-glutamine, L-glycyl-L-glutamine, N-acetyl-L-glutamine, or a combination thereof; and/or

lipid.

12. The method of any one of claims 1-11, wherein the perfusion culturing in step (d) comprises:

(i) adding an additional volume of the third culture medium to the third cell culture for a first period of time, and

(ii) adding an additional volume of a fourth culture medium to the third cell culture for a second period of time.

13. The method of any one of claims 1-12, wherein the third culture medium comprises one or more of: transferrin, insulin, SCF, and EPO.

14. The method of any one of claims 1-13, wherein the third culture medium comprises:

about 1 mM to about 8 mM of L-glutamine, L-alanyl-L-glutamine, L-glycyl-L-glutamine, N-acetyl-L-glutamine, or a combination thereof; and/or

about 0.5% v/v to about 10% v/v serum.

15. The method of any one of claims 12-14, wherein the fourth culture medium comprises one or more of: transferrin, insulin, and EPO.

16. The method of any one of claims 12-15, wherein the fourth culture medium comprises:

about 1 mM to about 8 mM of L-glutamine, L-alanyl-L-glutamine, L-glycyl-L-glutamine, N-acetyl-L-glutamine, or a combination thereof; and/or

about 0.5% v/v to about 10% v/v serum.

17. A method of generating a population of enucleated erythroid cells, the method comprising:

(a) disposing a volume of a first cell culture of erythroid progenitor cells into a second culture medium comprised within a vessel to provide a second cell culture with an initial cell density of about 0.1 x $10^5$ cells/mL to about 1 x $10^7$ cells/mL;

(b) batch or fed batch culturing the second cell culture for about 2 days to about 15 days;

(c) disposing a volume of the second cell culture of step (b) into a third culture medium comprised within a perfusion bioreactor to provide a third cell culture with an initial cell density of about 1 x $10^5$ to about 1 x $10^7$ cells/mL;

(d) perfusion culturing the third cell culture of step (c) for about 5 days to 20 days,

wherein after step (d) the third culture medium comprises a population of enucleated erythroid cells.

18. A method of generating a population of enucleated erythroid cells, the method comprising:

(a) disposing a volume of a first cell culture of erythroid progenitor cells into a second culture medium comprised within a perfusion bioreactor to provide a second cell culture with an initial cell density of about 0.1 x $10^5$ cells/mL to about 1 x $10^7$ cells/mL;

(b) perfusion culturing the second cell culture for about 2 days to about 15 days;

(c) disposing a volume of the second cell culture of step (b) into a third culture medium comprised within a vessel to provide a third cell culture with an initial cell density of about 1 x $10^5$ to about 1 x $10^7$ cells/mL;

(d) batch or fed batch culturing the third cell culture of step (c) for about 5 days to 20 days,

wherein after step (d) the third culture medium comprises a population of enucleated erythroid cells.

19. The method of any one of claims 1-18, wherein:

the method further comprises, prior to step (a), introducing a nucleic acid into the erythroid progenitor cells in the first cell culture, and

the method results in the production of a population of engineered enucleated erythroid cells.

20. The method of claim 19, wherein the population of engineered enucleated erythroid cells are engineered enucleated human erythroid cells.

21. The method of claim 19 or 20, wherein the engineered human enucleated erythroid cells comprise one or more exogenous protein(s).

22. The method of any one of claims 19-21, wherein the method further comprises:

(e) isolating the population of engineered enucleated erythroid cells from the third cell culture in step (d).

23. The method of claim 22, wherein the method further comprises:

(f) formulating the population of engineered enucleated erythroid cells isolated in step (e).

24. The method of claim 23, wherein the method further comprises:

(g) administering the formulated population of engineered enucleated erythroid cells in step (f) to a subject in need thereof.

25. A population of enucleated erythroid cells produced by the method of any one of claims 19-22.

26. A formulation produced by the method of claim 23.