CLAIMS

We claim:

1. A method of generating human embryonic mesenchymal progenitor (hEMP) cells, the method comprising the steps of:

(a) incubating clusters of stem cells with a Trypsin-like enzyme to generate non-clustered single stem cells;

(b) contacting non-clustered stem cells with a substrate coated with recombinant human vitronectin, but not mouse embryonic fibroblasts (MEFs) at a defined single cell density;

(c) cultivating the stem cells under culture conditions that facilitate cell growth to a desired confluence; and

(d) modifying the culture conditions by contacting the stem cells with a serum free media comprising BMP4, VEGF, and bFGF for a desired incubation time, and transiently exposing the stem cells to activin A to induce differentiation of the stem cells into hEMP cells;

thereby generating hEMP cells.

2. The method of claim 1, wherein the stem cells are human embryonic stem (ES) cells or induced pluripotent stem (iPS) cells.

3. The method of claim 2, wherein the ES or iPS cells are human in origin.

4. The method of claim 3, wherein the ES or iPS cells are H1 cells, H9 cells, HES3 cells, HSF1 cells, HSF6 cells, ESI-017 cells, CS02iCTR-NTn1 cells, CS03iCTR-NTn1 cells, CS80iCTR-Tn3 cells, CS179iCTR-NTn1 cells, CS201iCTR-NTn4 cells, CS202iCTR-NTn2 cells, or CS206iCTR-Tn5 cells.

5. The method of any one of the preceding claims, wherein the defined single cell density is between about $1.5x10^5$ and about $8x10^5$ cells/cm^2.

6. The method of claim 5, wherein the defined single cell density is about$1.89x10^5$ cells/cm^2, about $3.2x10^5$ cells/cm^2, about $3.4x10^5$ cells/cm^2, about $3.6\ x10^5$ cells/cm^2, about $3.79\ x10^5$ cells/cm^2, about $7.2x10^5$ cells/cm^2, or about $7.58\ x10^5$ cells/cm^2.

7. The method of any one of the preceding claims, wherein the substrate is a welled plate, a cell culture dish, a membrane, a bag, a culture flask, an inverse opal, a polymer lattice, a static cell suspension, an agitated cell suspension, or a plasma treated polymer.

8. The method of claim 7, wherein the substrate comprises a membrane.

9. The method of any one of the preceding claims, wherein the culture conditions that facilitate cell growth comprise cultivating the stem cells in mTeSR1 media.

10. The method of claim 9, wherein the mTeSR1 media comprises the ROCK inhibitor Y27632.

11. The method of any one of the preceding claims, wherein the desired confluence is between about 20% and about 80%.

12. The method of claim 11, wherein the confluence is about 20%, about 30%, about 40%, about 50%, about 60%, about 70% or about 80%.

13. The method of any one of the preceding claims, wherein the incubation time is between about 2 and about 4 days.

14. The method of claim 13, wherein the incubation time is about 2.0, about 2.5, about 3.0, about 3.5 or about 4.0 days.

15. The method of claim 14, wherein the incubation time is about 3.5 days.

16. The method of any one of the preceding claims, further comprising the step of differentiating the hEMP cells into T cells.

17. The method of claim 1, wherein the Trypsin-like enzyme is Trypsin, TrypLE Express, TrypLE Select, collagenase, dispase, Trypsin-EDTA.

18. A human embryonic mesenchymal progenitor (hEMP) cell generated according to a method of any one of the preceding claims.

19. A composition comprising a population of human embryonic mesenchymal progenitor (hEMP) cells generated according to a method of any one of claims 1-17.