human articular chondrocytes when cultured in vitro as well as the relative difficulty of re-differentiating the cells such that they produce abundant cartilage matrix at the graft site. Further, only a small amount of cartilage can be collected from humans, and thus only a small number of chondrocytes can be used for the initiation of the culture. Thus, there is continued difficulty in applying isolated human chondrocytes to transplantation therapy in practice.

Another therapeutic strategy is the utilization of bone marrow-derived mesenchymal stem cells (hbmMSCs). Clinical studies utilizing a single dose of hbmMSCs, (Chondrogen) show a reduction in pain compared to hyaluronic acid (HA) control. However, Mesenchymal stem cells (MSCs) have two hurdles in regard to their use in regenerating cartilage. First, the use of the cells as an allogeneic graft is problematic due to the limited proliferative capacity of adult MSCs, and even if the cells are capable of a certain amount of expansion, they often relatively quickly lose their capacity to form cartilage. The second hurdle is that MSCs, such as bone marrow-derived MSCs form hypertrophic chondrocytes, characterized by high levels of COL10A1 and IHH expression. The role of these chondrocytes in development are to recruit blood vessels and osteoblasts and then die. Hypertrophic chondrocytes are observed for instance in the growth plate regions of long bones. They are also observed at the site of a bone fracture where they similarly play an important role in bone formation. Therefore, the use of MSCs in the treatment of trauma or degenerative diseases of cartilage, such as osteoarthritis have yielded mixed results. In addition, there are numerous types of cartilage in the body. The elastic cartilage of the ear has differing molecular composition than that of the nose, sternum, trachea, and weight-bearing joints.

Therefore, the field of regenerative medicine, particularly in the field of cartilage regeneration and repair, are in great need of novel cellular formulations to generate commercial quantities of diverse types of permanent, as opposed to hypertrophic, chondrocytes.

## SUMMARY

Aspects of the present disclosure~~invention~~ include methods and compositions related to the production, identification and use of embryonic progenitor cell lines that are capable of undergoing chondrogenesis. A number of exemplary chondrogenic cell lines derived from primordial stem cells are disclosed. The chondrogenic cell lines described herein are robust, can expand for >40 passages, and have site-specific purity, thus providing for compositions and methods of producing diverse cartilage types with unique molecular compositions for use in research and therapy.

In one aspect, there is provided a method of producing cartilage, the method comprising culturing a clonally-purified embryonic progenitor cell line; wherein said cell line is negative for the expression of one or more of the genes selected from the group consisting of: CD74, CD90, CD166, ITGA2, and KCNK2; wherein said cell line is selected from the group consisting of: SM30, E15, 4D20.8, 7SM0032, MEL2, SK11 and combinations thereof; and wherein said culturing comprises culture under cartilage producing conditions for an amount of time effective to induce cartilage production from said cells.

WHAT IS CLAIMED IS:

1. A method of generating cartilage in a subject, the method comprising:

administering to the subject an effective amount of an embryonic progenitor cell line composition comprising:

(a) an embryonic progenitor cell line negative for the expression of one or more the genes selected from the group consisting of: CD74, CD90, CD166, ITGA2, KCNK2, and combinations thereof; and

(b) a pharmaceutically acceptable carrier.

2. The method of claim 1, wherein the embryonic progenitor cell line is negative for the expression of CD74.

3. The method of claim 2, wherein the embryonic progenitor cell line is further negative for the expression of one or more genes selected from the group consisting of: HOX genes and PITX1.

4. The method of claim 1, wherein the embryonic progenitor cell line generates cartilage in the absence of COL10A1 marker expression.

5. The method of claim 1, wherein the embryonic progenitor cell line is selected from the group consisting of: SM30, E15, 4D20.8, 7SMOO32, MEL2, SK11, 7PEND24 and 7SMOO7 and combinations thereof.

6. The method of claim 1, wherein the subject has cartilage damage and the method comprises administering the embryonic progenitor cell line composition to the site of said cartilage damage.

7. The method of claim 6, wherein the cartilage damage is articular cartilage damage.

8. The method of claim 1, wherein the pharmaceutically acceptable carrier comprises a composition selected from the group consisting of: hetastarch; hyaluronan and polymers thereof; chondroitin sulfate; type I collagen; type II collagen; type III collagen; polyanhydride,

polyorthoester, polyglycolic acid and copolymers thereof; alginate; agarose; polaxomers; fibrin; chitin; and chitosan and combinations thereof.

9. The method of claim 1, wherein the embryonic progenitor cell line is cultured under chondrocyte inducing conditions prior to administration to the subject.

10. The method of claim 1, further comprising measuring the rate of repair of the damaged cartilage over time.

11. A method of producing cartilage, the method comprising culturing a clonally-purified embryonic progenitor cell line negative for the expression of one or more the genes selected from the group consisting of: CD74, CD90, CD166, ITGA2, and KCNK2 under cartilage producing conditions.

12. The method of claim 11, wherein the embryonic progenitor cell line is negative for the expression of CD74.

13. The method of claim 12, wherein the embryonic progenitor cell line is further negative for the expression of one or more genes selected from the group consisting of: HOX genes and PITX1.

14. The method of claim 13, wherein the embryonic progenitor cell line generates cartilage in the absence of COL10A1 marker expression.

15. The method of claim 11, wherein the embryonic progenitor cell line is selected from the group consisting of: SM30, E15, 4D20.8, 7SMOO32, MEL2, SK11, 7PEND24 and 7SMOO7 and combinations thereof.

16. The method of claim 11, wherein the cartilage producing condition is selected from one or more of the group consisting of: chondrocyte culture conditions; impregnating the embryonic progenitor cell line into synthetic matrices or biological resorbable immobilization vehicles; and placing the embryonic progenitor cell line into a molded structure.

17. The method of claim 11, wherein the method further comprises transplanting the cartilage produced by the embryonic progenitor cell line to a subject.

18. A kit comprising:

(a) an embryonic progenitor cell line negative for the expression of one or more the genes selected from the group consisting of: CD74, CD90, CD166, ITGA2, and KCNK2; and

(b) reagents for inducing cartilage production from the cell line.

19. The kit of claim 18, wherein the embryonic progenitor cell line is selected from from the group consisting of: SM30, E15, 4D20.8, 7SMOO32, MEL2, SK11, 7PEND24 and 7SMOO7 and combinations thereof.

20. The kit of claim 18, wherein the reagents for inducing cartilage production comprise chondrocyte culture reagents.

21. A composition comprising:

chondrocyte progenitor cell line negative for the expression of one or more genes selected from the group consistin og: CD74, CD90, CD166, ITGA2, and KCNK2; and

a pharmaceutically acceptable carrier.

22. The composition of claim 21, wherein the chondrocyte progenitor cell line is negative for the expression of CD74.

23. The composition of claim 21, wherein said the chondrocyte progenitor cell line is further negative for the expression of one or more genes selected from the group consisting of: HOX genes and PITX1.

24. The composition of claim 21, wherein the chondrocyte progenitor cell line generates cartilage in the absence of COL10A1 marker expression.

25. The compositoin of claim 21, wherein the chondrocyte progenitor cell line is selected from the group consisting of: SM30, E15, 4D20.8, 7SMOO32, MEL2, SK11, 7PEND24 and 7SMOO7 and combinations thereof.

26. A chondrocyte progenitor cell line negative for the expression of one or more genes selected from the group consistin og: CD74, CD90, CD166, ITGA2, and KCNK2.

27. The cell line of claim 26, wherein the chondrocyte progenitor cell line is negative for the expression of CD74.

28. The cell line of claim 26, wherein said the chondrocyte progenitor cell line is further negative for the expression of one or more genes selected from the group consisting of: HOX genes and PITX1.

29. The cell line of claim 26, wherein the chondrocyte progenitor cell line generates cartilage in the absence of COL10A1 marker expression.

30. The cell line of claim 26, wherein the chondrocyte progenitor cell line is selected from the group consisting of: SM30, E15, 4D20.8, 7SMOO32, MEL2, SK11, 7PEND24 and 7SMOO7.

What is claimed is:

1. A method of producing cartilage, the method comprising culturing a clonally-purified embryonic progenitor cell line; wherein said cell line is negative for the expression of one or more of the genes selected from the group consisting of: CD74, CD90, CD166, ITGA2, and KCNK2; wherein said cell line is selected from the group consisting of: SM30, E15, 4D20.8, 7SM0032, MEL2, SK11 and combinations thereof; and wherein said culturing comprises culture under cartilage producing conditions for an amount of time effective to induce cartilage production from said cells.

2. The method of claim 1, wherein the embryonic progenitor cell line is negative for the expression of CD74.

3. The method of claim 2, wherein the embryonic progenitor cell line is further negative for the expression of one or more genes selected from the group consisting of: HOX genes and PITX1.

4. The method of claim 3, wherein the embryonic progenitor cell line generates cartilage in the absence of COL10A1 marker expression.

5. The method of claim 1, wherein the cartilage producing condition is selected from one or more of the group consisting of: chondrocyte culture conditions; impregnating the embryonic progenitor cell line into synthetic matrices or biological resorbable immobilization vehicles; and placing the embryonic progenitor cell line into a molded structure.