the use of MSCs, grown in these conditions for supporting *in vivo* engraftment of HSCs following HSC transplantation has never been suggested in any of these studies. Also, time consuming optimization of various conditions e.g., perfusion conditions, or various isolation techniques for specific cell types were required.

The use of a perfused Post-partum placenta as a 3D reactor for culturing MSCs was suggested in US Pat. No. 7045148 and U.S. Pat. App. Nos. 20020123141 20030032179 and 2005011871. However, this procedure is limited for up to 24 hours after the placenta is isolated and involves perfusion, therefore mass growth of the cells and its maintenance for prolonged time periods is not possible.

There is thus a widely recognized need for, and it would be highly advantageous to have, novel methods of cell expansion and uses of cells and conditioned medium produced thereby for therapy and which are devoid of the above limitations.

SUMMARY ~~OF THE INVENTION~~

Disclosed is~~According to one aspect of the present invention there is provided~~ a method of cell expansion, the method comprising culturing adherent cells from placenta or adipose tissue under three-dimensional culturing conditions, which support cell expansion.

In one aspect, there is provided a method of expanding a population of adherent undifferentiated stromal cells, comprising: (i) obtaining adherent stromal cells from human placenta or adipose tissue; (ii) inoculating a three-dimensional cell culture system comprising a culture medium comprising serum with the adherent undifferentiated stromal cells; (iii) culturing the adherent undifferentiated stromal cells under conditions that support expansion of the cells; and (iv) recovering the expanded adherent undifferentiated stromal cells from the three-dimensional culture system.

According to one embodiment~~another aspect~~ of the present disclosure~~invention~~ there is provided a method of producing a conditioned medium, the method comprising: culturing adherent cells from a placenta or adipose tissue in three dimensional culturing conditions which allow cell expansion; and collecting a conditioned medium of the expanded adherent cells, thereby producing the conditioned medium.

In one aspect, there is provided a population of expanded adherent undifferentiated stromal cells made according to the method as defined herein.

According to yet another embodiment~~aspect~~ of the present disclosure~~invention~~ there is provided a population of cells generated according to the method as above.

In one aspect, there is provided an isolated population of cells comprising adherent undifferentiated stromal cells of placenta or adipose tissue grown in a culture medium under three-dimensional culturing conditions, wherein said culture medium comprises serum, and said adherent undifferentiated stromal cells are characterized by at least one of: (i) secretion of a higher level of at least one factor selected from the group consisting of SCF, IL-6, and Flt-3 than that secreted by adherent cells of placenta or adipose tissue grown in a 2D culture; (ii) expression of a higher level of at least one protein selected from the group consisting of H2A histone family (H2AF), Aldehyde dehydrogenase X (ALDH X), eukaryotic translation elongation factor 2 (EEEF2), reticulocalbin 3, EF-hand calcium binding domain (RCN2) and calponin 1 basic smooth muscle (CNN1) than that expressed by adherent cells of placenta or adipose tissue grown in a 2D culture; (iii) expression of a lower level of at least one protein selected from the group consisting of heterogeneous nuclear ribonucleoprotein HI (Hnrph1), CD44 antigen isoform 2 precursor, 3 phosphoadenosine 5 phosphosulfate synthesis 2 isoform a (Papss2) and ribosomal protein L7a (rpL7a) than that expressed by adherent cells of placenta or adipose tissue grown in a 2D culture; and (iv) immunosuppressive activity.

According to still another embodimentaspect of the present disclosureinvention there is provided an isolated population of cells comprising adherent cells of placenta or adipose tissue, wherein the adherent cells secrete a higher level of at least one factor selected from the group consisting of SCF, IL-6, and Flt-3 than that secreted by adherent cells of placenta or adipose tissue grown in a 2D culture.

According to an additional embodimentaspect of the present disclosureinvention there is provided an isolated population of cells comprising adherent cells of placenta or adipose tissue, wherein the adherent cells express a higher level of at least one protein selected from

the group consisting of H2A histone family (H2AF), Aldehyde dehydrogenase X (ALDH X), eukaryotic translation elongation factor 2 (EEEF2), reticulocalbin 3, EF-hand calcium binding domain (RCN2) and calponin 1 basic smooth muscle (CNN1) than that expressed by adherent cells of placenta or adipose tissue grown in a 2D culture.

According to yet an additional embodimentaspect of the present disclosureinvention there is provided an isolated population of cells comprising adherent cells of placenta or adipose tissue, wherein the adherent cells express a lower level of expression of at least one protein selected from the group consisting of heterogeneous nuclear ribonucleoprotein H1 (Hnrph1), CD44 antigen isoform 2 precursor, 3 phosphoadenosine 5 phosphosulfate synthase 2 isoform a (Papss2) and ribosomal protein L7a (rpL7a) than that expressed by adherent cells of placenta or adipose tissue grown in a 2D culture.

According to still an additional embodimentaspect of the present disclosureinvention there is provided an isolated population of cells comprising adherent cells of placenta or adipose tissue, wherein the adherent cells are characterized by a higher immunosuppressive activity than that of adherent cells of placenta or adipose tissue grown in a 2D culture.

According to further features in preferred embodiments of the disclosureinvention described below the immunosuppressive activity comprises reduction in T cell proliferation.

In one aspect, there is provided a conditioned culture medium made according to the method as defined herein.

According to further embodimentaspect of the present disclosureinvention there is provided a pharmaceutical composition comprising, as an active ingredient, the population of cells generated according to the method as above.

In one aspect, there is provided a pharmaceutical composition comprising the adherent undifferentiated stromal cells as defined herein and a pharmaceutically acceptable carrier.

According to a further embodimentaspect of the present disclosureinvention there is provided a pharmaceutical composition comprising, as an active ingredient, the conditioned medium produced according to the method as above.

According to yet a further embodimentaspect of the present disclosureinvention there is provided a pharmaceutical composition comprising, as an active ingredient, the isolated population of cells according to above.

In one aspect, there is provided a pharmaceutical composition comprising the cells as defined herein and a pharmaceutically acceptable carrier.

According to still a further embodimentaspect of the present disclosureinvention there is provided a method of treating a condition which may benefit from stromal cell transplantation in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of adherent cells of a tissue selected from the group

consisting of placenta and adipose tissue, thereby treating the condition which may benefit from stem cell transplantation in the subject.

According to still a further embodimentaspect of the present disclosureinvention there is provided a method of treating a condition which may benefit from stromal cell transplantation in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a conditioned medium of adherent cells derived from a tissue selected from the group consisting of placenta and adipose tissue, thereby treating the condition which may benefit from stem cell transplantation in the subject.

According to still a further embodimentaspect of the present disclosureinvention there is provided a method of reducing an immune response in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the isolated population of cells of claims 3, 4, 5, 6 or 7, so as to reduce the immune response in the subject.

According to still further features in the described preferred embodiments the subject is treated with cell therapy.

According to still further features in the described preferred embodiments the method further comprises administering stem cells.

According to still further features in the described preferred embodiments the stem cells comprise hematopoietic stem cells.

According to still further features in the described preferred embodiments the cells are administered concomitantly with the conditioned medium or adherent cells.

According to still further features in the described preferred embodiments the cells are administered following administration of the conditioned medium or adherent cells.

According to still further features in the described preferred embodiments the adherent cells are obtained from a three dimensional culture.

According to still further features in the described preferred embodiments the adherent cells are obtained from a two dimensional culture.

According to still further features in the described preferred embodiments the condition is selected from the group consisting of stem cell deficiency, heart disease, Parkinson's disease, cancer, Alzheimer's disease, stroke, burns, loss of tissue, loss of blood, anemia, autoimmune disorders, diabetes, arthritis, Multiple Sclerosis, graft vs. host disease (GvHD), neurodegenerative disorders, autoimmune encephalomyelitis

consisting of SCF, Flt-3 and IL-6 higher than that secreted by adherent cells from placenta or adipose tissue grown in a 2D culture.

According to still further features in the described preferred embodiments the adherent cells express a higher level of at least one protein selected from the group consisting of H2A histone family (H2AF), Aldehyde dehydrogenase X (ALDH X), eukaryotic translation elongation factor 2 (EEEF2), reticulocalbin 3, EF-hand calcium binding domain (RCN2) and calponin 1 basic smooth muscle (CNN1) than that secreted by adherent cells from placenta or adipose tissue grown in a 2D culture.

According to still further features in the described preferred embodiments the adherent cells express a lower level of expression of at least one protein selected from the group consisting of heterogeneous nuclear ribonucleoprotein H1 (Hnrph1), CD44 ~~antigen~~ isoform 2 precursor, 3 phosphoadenosine 5 phosphosulfate synthase 2 isoform a (Papss2) and ribosomal protein L7a (rpL7a) than that secreted by adherent cells from placenta or adipose tissue grown in a 2D culture.

According to still further features in the described preferred embodiments the adherent cells or medium are characterized by a higher immunosuppressive activity than that of adherent cells of placenta or adipose tissue grown in a 2D culture.

According to still further features in the described preferred embodiments the immunosuppressive activity comprises reduction in T cell proliferation.

According to still further features in the described preferred embodiments the cells comprise cells having a stromal stem cell phenotype.

According to still further features in the described preferred embodiments the stromal stem cell phenotype comprises T cell suppression activity.

According to still further features in the described preferred embodiments thstromal stem cell phenotype comprises hematopoietic stem cell support activity.

According to still further features in the described preferred embodiments the use of the population of cells described above is for manufacture of a medicament identified for transplantation.

The present disclosure~~invention~~ successfully addresses the shortcomings of the presently known configurations by providing novel methods of cell expansion and uses of cells and conditioned medium produced thereby for therapy.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure~~invention~~ belongs. Although methods and materials similar or equivalent to those

described herein can be used in the practice or testing of the present disclosure~~invention~~, suitable methods and materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

FIGs. 1a-g depicts the bone-like microenvironment created in the bioreactor system containing 3-D carriers. Figures 1a-b are electron micrographs depicting the comparison of natural bone (Figure 1a) and the structure of the PluriX™ 3D carrier 7 days after seeding Adherent Stromal Cells (3D-ASC), imitating the bone micro-environment (Figure 1b). Figures 1c-f are electron micrographs depicting the PluriX™ 3D matrix seeded with 3D-ASC, produced from bone marrow, 20 days (Figures 1c-d, magnified X 150 and 250 respectively) and 40 days (Figures 1e-f, magnified X 350 and 500 respectively) after seeding. Figure 1g is a diagram of the Plurix 3D plug flow bioreactor with separate parts defined by numbers: Culture medium reservoir (1), gas mixture supply (2), filter (3), injection point (4), column in which the 3D carriers are placed (5) flow monitor (6), flow valve (6a), separating container (7), cell growth analyzers (8); peristaltic pump (9), sampling point (10), dissolved O_2 measurement electrode (11), pH measurement electrode (12), control system (13), fresh growth media (14), used growth media (15).

FIG. 2 is a graph depicting different production lots of adherent stromal cells (3D-ASC; Lots 5-8) originating from placenta, grown in 3D growth conditions within the bioreactor systems. ASCs (2×10^6) were seeded in the bioreactor at a density of

WHAT IS CLAIMED IS:

1. A method of cell expansion, the method comprising culturing adherent cells from placenta or adipose tissue under three-dimensional culturing conditions, which support cell expansion.

2. A method of producing a conditioned medium, the method comprising

(a) culturing adherent cells from a placenta or adipose tissue in three dimensional culturing conditions which allow cell expansion; and

(b) collecting a conditioned medium of said expanded adherent cells, thereby producing the conditioned medium.

3. A population of cells generated according to the method of claim 1.

4. An isolated population of cells comprising adherent cells of placenta or adipose tissue, wherein said adherent cells secrete a higher level of at least one factor selected from the group consisting of SCF, IL-6, and Flt-3 than that secreted by adherent cells of placenta or adipose tissue grown in a 2D culture.

5. An isolated population of cells comprising adherent cells of placenta or adipose tissue, wherein said adherent cells express a higher level of at least one protein selected from the group consisting of H2A histone family (H2AF), Aldehyde dehydrogenase X (ALDH X), eukaryotic translation elongation factor 2 (EEEF2), reticulocalbin 3, EF-hand calcium binding domain (RCN2) and calponin 1 basic smooth muscle (CNN1) than that expressed by adherent cells of placenta or adipose tissue grown in a 2D culture.

6. An isolated population of cells comprising adherent cells of placenta or adipose tissue, wherein said adherent cells express a lower level of expression of at least one protein selected from the group consisting of heterogeneous nuclear ribonucleoprotein H1 (Hnrph1), CD44 antigen isoform 2 precursor, 3 phosphoadenosine 5 phosphosulfate synthase 2 isoform a (Papss2) and ribosomal protein L7a (rpL7a) than that expressed by adherent cells of placenta or adipose tissue grown in a 2D culture.

7. An isolated population of cells comprising adherent cells of placenta or adipose tissue, wherein said adherent cells are characterized by a higher immunosuppressive activity than that of adherent cells of placenta or adipose tissue grown in a 2D culture.

8. The isolated population of cells of claim 7, wherein said immunosuppressive activity comprises reduction in T cell proliferation.

9. A pharmaceutical composition comprising, as an active ingredient, the population of cells generated according to claim 1.

10. A pharmaceutical composition comprising, as an active ingredient, the conditioned medium produced according to claim 2.

11. A pharmaceutical composition comprising, as an active ingredient, the isolated population of cells of claims 4, 5, 6 or 7.

12. A method of treating a condition which may benefit from stromal cell transplantation in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of adherent cells of a tissue selected from the group consisting of placenta and adipose tissue, thereby treating the condition which may benefit from stem cell transplantation in the subject.

13. A method of treating a condition which may benefit from stromal cell transplantation in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a conditioned medium of adherent cells derived from a tissue selected from the group consisting of placenta and adipose tissue, thereby treating the condition which may benefit from stem cell transplantation in the subject.

14. A method of reducing an immune response in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the isolated population of cells of claims 3, 4, 5, 6 or 7, so as to reduce the immune response in the subject.

15. The method of claim 14, wherein the subject is treated with cell therapy.

16. The method of claim 12 or 13, further comprising administering stem cells.

17. The method of claim 16, wherein said stem cells comprise hematopoietic stem cells.

18. The method of claim 16, wherein said stem cells are administered concomitantly with said conditioned medium or adherent cells.

19. The method of claim 16, wherein said stem cells are administered following administration of said conditioned medium or adherent cells.

20. The method of claims 12 and 13, wherein said adherent cells are obtained from a three dimensional culture.

21. The method of claims 12 and 13, wherein said adherent cells are obtained from a two dimensional culture.

22. The method of claims 12 and 13, wherein said condition is selected from the group consisting of stem cell deficiency, heart disease, Parkinson's disease, cancer, Alzheimer's disease, stroke, burns, loss of tissue, loss of blood, anemia, autoimmune disorders, diabetes, arthritis, Multiple Sclerosis, graft vs. host disease (GvHD), neurodegenerative disorders, autoimmune encephalomyelitis (EAE), systemic lupus erythematosus (SLE), rheumatoid arthritis, systemic sclerosis, Sjorgen's syndrome, multiple sclerosis (MS), Myasthenia Gravis (MG), Guillain-Barré Syndrome (GBS), Hashimoto's Thyroiditis (HT), Graves's Disease, Insulin dependent Diabetes Melitus (IDDM) and Inflammatory Bowel Disease.

23. The method or cell population of any of claims 1, 2, 3 or 20, wherein said three dimensional culture comprises a 3D bioreactor.

24. The method of claim 23, wherein said bioreactor is selected from the group consisting of a plug flow bioreactor, a continuous stirred tank bioreactor and a stationary-bed bioreactor.

25. The method or cell population of any of claims 1, 2, 3 or 20, wherein said culturing of said cells is effected under a continuous flow of a culture medium.

26. The method or cell population of any of claims 1, 2, 3 or 20, wherein said three dimensional culture comprises an adherent material selected from the group consisting of a polyester, a polyalkylene, a polyfluorochloroethylene, a polyvinyl chloride, a polystyrene, a polysulfone, a cellulose acetate, a glass fiber, a ceramic particle, a matrigel, an extracellular matrix component, a collagen, a poly L lactic acid and an inert metal fiber.

27. - The method or cell population of any of claims 1, 2, 3, or 20, wherein said culturing is effected for at least 3 days.

28. The method or cell population of claim 21, wherein said culturing is effected for at least 3 days.

29. The method or cell population of any of claims 1, 2, 3 or 20, wherein said culturing is effected until said adherent cells reach at least 60 % confluence.

30. The methods of claims 12 and 13, wherein the condition may benefit from the facilitation of hematopoietic stem cell engraftment.

31. The methods, population of cells or medium of claims 1, 2, 3, 4, 5, 6, 7 or 20, wherein said adherent cells comprise a positive marker expression array selected from the group consisting of CD73, CD90, CD29 and CD105.

32. The methods, population of cells or medium of claims 1, 2, 3, 4, 5, 6, 7 or 20, wherein said adherent cells comprise a negative marker expression array selected from the group consisting of CD45, CD80, HLA-DR, CD11b, CD14, CD19, CD34 and CD79.

33. The methods, population of cells or medium of claims 1, 2, 3, or 20, wherein said adherent cells secrete a higher level of at least one factor selected from the group consisting of SCF, Flt-3 and IL-6 higher than that secreted by adherent cells from placenta or adipose tissue grown in a 2D culture.

34. The methods, population of cells or medium of claims 1, 2, 3, or 20, wherein said adherent cells express a higher level of at least one protein selected from the group consisting of H2A histone family (H2AF), Aldehyde dehydrogenase X (ALDH X), eukaryotic translation elongation factor 2 (EEEF2), reticulocalbin 3, EF-hand calcium binding domain (RCN2) and calponin 1 basic smooth muscle (CNN1) than that secreted by adherent cells from placenta or adipose tissue grown in a 2D culture.

35. The methods, population of cells or medium of claims 1, 2, 3, or 20, wherein said adherent cells express a lower level of expression of at least one protein selected from the group consisting of heterogeneous nuclear ribonucleoprotein H1 (Hnrph1), CD44 antigen isoform 2 precursor, 3 phosphoadenosine 5 phosphosulfate synthase 2 isoform a (Papss2) and ribosomal protein L7a (rpL7a) than that secreted by adherent cells from placenta or adipose tissue grown in a 2D culture.

36. The methods, population of cells or medium of claims 1, 2, 3, or 20, wherein said adherent cells or medium are characterized by a higher immunosuppressive activity than that of adherent cells of placenta or adipose tissue grown in a 2D culture.

37. The isolated population of cells of claim 36, wherein said immunosuppressive activity comprises reduction in T cell proliferation.

38. The method or cell population of any of claims 1, 2, 3, 4, 5, 6, 7, 12, or 13, wherein said cells comprise cells having a stromal stem cell phenotype.

39. The method of claim 38, wherein said stromal stem cell phenotype comprises T cell suppression activity.

40. The method of claim 38, wherein said stromal stem cell phenotype comprises hematopoietic stem cell support activity.

41. Use of the population of cells of claim 3, 4, 5, 6, or 7 for manufacture of a medicament identified for transplantation.

WHAT IS CLAIMED IS:

1. A method of expanding a population of adherent undifferentiated stromal cells, comprising:

(i) obtaining adherent stromal cells from human placenta or adipose tissue;

(ii) inoculating a three-dimensional cell culture system comprising a culture medium comprising serum with the adherent undifferentiated stromal cells;

(iii) culturing the adherent undifferentiated stromal cells under conditions that support expansion of the cells; and

(iv) recovering the expanded adherent undifferentiated stromal cells from the three-dimensional culture system.

2. The method of claim 1, further comprising collecting the culture medium in which the expanded adherent undifferentiated stromal cells are cultured, thereby producing a conditioned culture medium comprising Flt-3 ligand, IL-6, and SCF.

3. The method of claim 1 or claim 2, wherein the three-dimensional culture system comprises a three-dimensional bioreactor.

4. The method of any one of claims 1 to 3, wherein the adherent undifferentiated stromal cells are cultured to at least 60% confluence.

5. A population of expanded adherent undifferentiated stromal cells made according to the method of claim 1 or claim 2.

6. The population of expanded adherent undifferentiated stromal cells of claim 5, wherein the cells suppress an immune reaction.

7. A conditioned culture medium made according to the method of claim 2.

8. A pharmaceutical composition comprising the adherent undifferentiated stromal cells of claim 5 or claim 6 and a pharmaceutically acceptable carrier.

9. An isolated population of cells comprising adherent undifferentiated stromal cells of placenta or adipose tissue grown in a culture medium under three-dimensional culturing conditions, wherein said culture medium comprises serum, and said adherent undifferentiated stromal cells are characterized by at least one of:

(i) secretion of a higher level of at least one factor selected from the group consisting of SCF, IL-6, and Flt-3 than that secreted by adherent cells of placenta or adipose tissue grown in a 2D culture;

(ii) expression of a higher level of at least one protein selected from the group consisting of H2A histone family (H2AF), Aldehyde dehydrogenase X (ALDH X), eukaryotic translation elongation factor 2 (EEEF2), reticulocalbin 3, EF-hand calcium binding domain (RCN2) and calponin 1 basic smooth muscle (CNN1) than that expressed by adherent cells of placenta or adipose tissue grown in a 2D culture;

(iii) expression of a lower level of at least one protein selected from the group consisting of heterogeneous nuclear ribonucleoprotein HI (Hnrph1), CD44 antigen

isoform 2 precursor, 3 phosphoadenosine 5 phosphosulfate synthase 2 isoform a (Papss2) and ribosomal protein L7a (rpL7a) than that expressed by adherent cells of placenta or adipose tissue grown in a 2D culture; and

(iv) immunosuppressive activity.

10. The isolated population of cells of claim 9, wherein said immunosuppressive activity comprises reduction in T cell proliferation.

11. A pharmaceutical composition comprising the cells of claim 9 and a pharmaceutically acceptable carrier.