Endothelial cells were also maintained in EGM-2MV media as positive control. A, HUVECs. B, HCAECs. C, HIAECs.

[0025] Figure 2 shows the effect of hUTC lot#120304 and neutralizing antibodies on the proliferation of endothelial cells. HUVECs or HCAECs were seeded onto the bottom of a 24-well tissue culture dish at a density of 5000 cells/cm^2 (10,000 cells/well) and hUTC lot#120304 inside transwell inserts at a density of 5000 cells/cm^2 (1,650 cells/insert) in co-culture media (Hayflick 50% + EGM-2MV 50%). Neutralizing antibodies to FGF (7 μg/ml), HGF (1 μg/ml), or VEGF (1μg/ml) were also added at this time. After 7 days of co-culture, cells were harvested and counted using a Guava® instrument. Endothelial cells were also maintained in EGM-2MV media as positive control. Cells treated with growth factor alone and growth factor plus neutralizing antibodies are shown. A and B, HUVECs. C and D, HCAECs.

[0026] Figure 3 shows the effect of hUTC lot#120304 cell lysate and neutralizing antibodies on proliferation of HUVECs. HUVECs were seeded onto the bottom of a 24-well tissue culture dish at a density of 5000 cells/cm^2 (10,000 cells/well) in EGM-2MV media for 8h. Cells were then serum-starved by overnight incubation in 0.5 ml of EGM-2MV media containing 0.5% FBS and without growth factors. Afterwards, FBS, freshly prepared hUTC lot#120304 cell lysates, and neutralizing antibodies to FGF (7 μg/ml) or HGF (1 μg/ml) were added. After 4 days of culture, cells were harvested and counted using a Guava® instrument. Light grey bars, media controls. Medium grey bars, HUVECs incubated with lysate containing 62.5 μg of protein. Dark grey bars, HUVECs incubated with lysate containing 125 μg of protein.

[0027] Figure 4 shows the effect of hUTCs and MSCs on the migration of endothelial cells. HUVECs or HCAECs were seeded inside transwell inserts at a density of 5000 cells/cm^2 (23,000 cells/insert) and hUTC lot#120304 or MSCs onto the bottom of a 6-well tissue culture dish at a density of 5000 cells/cm^2 (48,000 cells/well) in co-culture media (Hayflick 50% + EGM-2MV 50%). After 7 days of co-culture, cells that were on the underside of the transwell insert were harvested and counted using a Guava® instrument. Endothelial cells were also maintained in EGM-2MV media as control. A, HUVECs. B, HCAECs.

[0028] Figure 5 shows the effect of hUTC lot#120304 and neutralizing antibodies on the migration of endothelial cells. HUVECs or HCAECs were seeded inside transwell inserts at a density of 5000 cells/cm^2 (23,000 cells/insert) and hUTC lot#120304 onto the

[0185] The effect of hUTC lot#120304 on the proliferation of endothelial cells cultured in H80 compared with H50 is shown in Figure 1. The proliferation of HUVECs maintained in H50 was higher than those kept in H80 (Figure 1A) while HCAECs and HIAECs exhibited similar growth in these co-culture media formulations (Figure 1B and Figure 1C). In both media formulations, co-culture of endothelial cells with hUTC lot#120304 resulted in significant increases in cell number after 7 days. All subsequent co-culture studies of hUTCs and endothelial cells were performed in the Hayflick 50% + EGM-2MV 50% (H50) media formulation.

[0186] MSCs and fibroblasts were also tested in co-cultures with endothelial cells to determine whether other cell types have the ability to influence the proliferation of endothelial cells. As shown in Figure 1A, there was no difference in the proliferation of HUVECs in co-culture media (H50 or H80) and those that were co-cultured with MSCs or with fibroblasts. The same was true of HCAECs (Figure 1B) and HIAECs (Figure 1C) where co-culture with hUTC lot#120304 resulted in increased cell proliferation while no differences can be observed between cells in co-culture media (H50 or H80) and those that were co-cultured with MSCs.

[0187] To investigate the potential mechanisms of hUTC-mediated increase in endothelial cell proliferation, neutralizing antibodies to FGF (7 μg/ml), HGF (1 μg/ml), and VEGF (1μg/ml) were included in co-cultures of HUVECs and HCAECs with hUTCs. Results in Figures 2A to 2D show that in both HUVECs and HCAECs the addition of neutralizing antibodies to FGF and HGF reduced the increase in cell number induced by hUTC lot#120304. At the concentrations that were used for these studies, these neutralizing antibodies blocked proliferation of HUVECs induced by the growth factors (Figures 2A and 2B). It is interesting to note that a neutralizing antibody to VEGF did not have a significant effect on the cell proliferation induced by co-culture of both HUVECs (Figures 2A and 2B) and HCAECs (Figures 2C and 2D) with hUTC lot#120304. In separate studies, the proliferation of hUTC lot#120304 was not affected by the addition of neutralizing antibodies to FGF and VEGF to the culture media (data not shown).

Effect of hUTC lot#120304 cell lysate on proliferation of HUVECs

[0188] Studies were also conducted to determine the effect of cell lysate on the proliferation of HUVECs. HUVECs were seeded onto 24-well plates in EGM-2MV media for 8h at a density of 5000 cells/cm^2. The cells were then serum-starved by an overnight incubation in 0.5 ml of EGM-2MV media containing 0.5% fetal bovine serum (FBS) and

What is claimed is:

1. Use of fibrin glue and an isolated homogenous population of cells obtained from human umbilical cord tissue in the manufacture of a medicament for the treatment of a peripheral vascular disease, wherein the umbilical cord tissue is substantially free of blood, and wherein said isolated homogenous population of the cells is capable of self-renewal and expansion in culture, has the potential to differentiate and does not express CD117 and/or telomerase.

2. The use of claim 1, wherein the isolated population of cells has one or more of the following characteristics:

(a) expresses oxidized low density lipoprotein receptor 1, reticulon, chemokine receptor ligand 3, and/or granulocyte chemotactic protein;

(b) does not express CD31, CD34 or CD45;

(c) expresses, relative to a human fibroblast, mesenchymal stem cell, or iliac crest bone marrow cell, increased levels of interleukin 8 or reticulon 1;

(d) has the potential to differentiate into cells of at least a skeletal muscle, vascular smooth muscle, pericyte or vascular endothelium phenotype; and

(e) expresses CD10, CD13, CD44, CD73, and CD90.

3. The use of claims 1 or 2, wherein the peripheral vascular disease is peripheral ischemia.

4. The use of claim 3, wherein the use comprises administration at the sites of peripheral ischemia.

5. The use of claim 3, wherein the use comprises administration by intramuscular injection, intravascular injection or injection into adipose depots in muscle.

6. The use of claims 1 or 2, wherein the use comprises local administration.

7. The use of claims 1 or 2, wherein the use comprises administration by injection, infusion, a device implanted in a patient, or by implantation of a matrix or scaffold containing the medicament.

8. The use of claims 1 or 2, wherein the isolated population of cells is induced *in vitro* to differentiate into a skeletal muscle, vascular muscle, perictye or vascular endothelium lineage prior to administration.

9. The use of any of claims 1 to 7, wherein the population of cells is genetically engineered to produce a gene product that promotes treatment of peripheral vascular disease.

10. The use of any of claims 1 to 9, wherein the medicament further comprises an agent selected from the group consisting of an antithrombogenic agent, an immunosuppressive agent, an immunomodulatory agent, a pro-angiogenic, an antiapoptotic agent and mixtures thereof.

11. The use of any of claims 1 to 10, wherein the medicament further comprises at least one other cell type.

12. The use of claim 11, wherein the other cell type is a skeletal muscle cell, a skeletal muscle progenitor cell, a vascular smooth muscle cell, a vascular smooth muscle progenitor cell, a pericyte, a vascular endothelial cell, a vascular endothelium progenitor cell or other multipotent or pluripotent stem cell.

13. The use of any of claims 1 to 12, wherein the use comprises exertion of a trophic effect.

14. The use of claim 14, wherein the trophic effect is proliferation of vascular endothelial cells.

15. The use of any of claims 1 to 14, wherein the use induces migration of vascular endothelial cells and/or vascular endothelium progenitor cells to the sites of the peripheral disease.

16. The use of any of claims 1 to 15, wherein the use induces migration of vascular smooth muscle cells and/or vascular smooth muscle progenitor cells to the sites of the peripheral disease.

17. The use of any of claims 1 to 16, wherein the use induces migration of pericytes to the sites of the peripheral vascular disease.

18. The use of any of claims 1 to 17, wherein the fibrin glue comprises fibrinogen and thrombin.

19. The use of claim 18, wherein the fibrin glue comprises from about 16 to about 24 IU/ml of thrombin and from about 39.3 to about 60.7 mg/ml of fibrinogen.

20. A kit for treating a patient having a peripheral vascular disease comprising fibrinogen, thrombin and an isolated homogenous population of cells obtained from human umbilical cord tissue in an amount effective to treat the disease, wherein the umbilical cord tissue is substantially free of blood, and wherein said isolated homogenous population of the cells is capable of self-renewal and expansion in culture, has the potential to differentiate and do not express CD117 and/or telomerase.

21. The kit of claim 20 further comprising instructions for use.

22. The kit of claims 20 or 21, wherein fibrinogen and the isolated homogenous population of cells are in a composition.

23. The kit of claim 22, wherein thrombin is added to the composition immediately prior to use.

24. The kit of claim 20, wherein the kit comprises from about 16 to about 24 IU/ml of thrombin and from about 39.3 to about 60.7 mg/ml of fibrinogen.

25. The kit of any of claims 20 to 24, wherein the isolated population of cells has one or more of the following characteristics:

(a) expresses oxidized low density lipoprotein receptor 1, reticulon, chemokine receptor ligand 3, and/or granulocyte chemotactic protein;

(b) does not express CD31, CD34 or CD45;

(c) expresses, relative to a human fibroblast, mesenchymal stem cell, or iliac crest bone marrow cell, increased levels of interleukin 8 or reticulon 1;

(d) has the potential to differentiate into cells of at least a skeletal muscle, vascular smooth muscle, pericyte or vascular endothelium phenotype; and

(e) expresses CD10, CD13, CD44, CD73, and CD90.

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It is interesting to note that a neutralizing antibody to VEGF did not have a significant effect on the cell proliferation induced by co-culture of both HUVECs (Figure 2A) and HCAECs (Figure 2B) with hUTC lot#120304. In separate studies, the proliferation of hUTC lot#120304 was not affected by the addition of neutralizing antibodies to FGF and VEGF to the culture media (data not shown).

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Claims

1. Use of fibrin glue and an isolated homogenous population of cells obtained from human umbilical cord tissue in the manufacture of a medicament for enhancing reperfusion in a patient having peripheral ischemia, wherein the umbilical cord tissue is substantially free of blood, and wherein said isolated homogenous population of the cells is capable of self-renewal and expansion in culture, has the potential to differentiate and does not express CD117 and/or telomerase.

2. The use of claim 1, wherein the isolated population of cells has one or more of the following characteristics:

(a) expresses oxidized low density lipoprotein receptor 1, reticulon, chemokine receptor ligand 3, and/or granulocyte chemotactic protein;

(b) does not express CD31, CD34 or CD45;

(c) expresses, relative to a human fibroblast, mesenchymal stem cell, or iliac crest bone marrow cell, increased levels of interleukin 8 or reticulon 1;

(d) has the potential to differentiate into cells of at least a skeletal muscle, vascular smooth muscle, pericyte or vascular endothelium phenotype; and

(e) expresses CD10, CD13, CD44, CD73, and CD90.

3. The use of claims 1 or 2, wherein the medicament is formulated for administration at the sites of peripheral ischemia.

4. The use of claims 1 or 2, wherein the medicament is formulated for administration by intramuscular injection, intravascular injection or injection into adipose depots in muscle.

5. The use of claims 1 or 2, wherein the medicament is formulated for local administration.

6. The use of claims 1 or 2, wherein the isolated population of cells is induced *in vitro* to differentiate into a skeletal muscle, vascular muscle, perictye or vascular endothelium lineage prior to administration.

7. The use of any of claims 1 to 5, wherein the population of cells is genetically engineered to produce a gene product that promotes treatment of peripheral vascular disease.

8. The use of any of claims 1 to 5, 6 and 7, wherein the medicament further comprises an agent selected from the group consisting of an antithrombogenic agent, an immunosuppressive agent, an immunomodulatory agent, a pro-angiogenic, an antiapoptotic agent and mixtures thereof.

9. The use of any of claims 1 to 5 and 6 to 8, wherein the medicament further comprises at least one other cell type.

10. The use of claim 9, wherein the other cell type is a skeletal muscle cell, a skeletal muscle progenitor cell, a vascular smooth muscle cell, a vascular smooth muscle progenitor cell, a pericyte, a vascular endothelial cell, a vascular endothelium progenitor cell or other multipotent or pluripotent stem cell.

11. The use of any of claims 1 to 5 and 6 to 10, wherein the use comprises exertion of a trophic effect.

12. The use of claim 11, wherein the trophic effect is proliferation of vascular endothelial cells.

13. The use of any of claims 1 to 5 and 6 to 12, wherein the use induces migration of vascular endothelial cells and/or vascular endothelium progenitor cells to the sites of the peripheral disease.

14. The use of any of claims 1 to 13, wherein the use induces migration of vascular smooth muscle cells and/or vascular smooth muscle progenitor cells to the sites of the peripheral disease.

15. The use of any of claims 1 to 5 and 6 to 14, wherein the use induces migration of pericytes to the sites of the peripheral vascular disease.

16. The use of any of claims 1 to 5 and 6 to 15, wherein the fibrin glue comprises fibrinogen and thrombin.

17. The use of claim 16, wherein the fibrin glue comprises from about 16 to about 24 IU/ml of thrombin and from about 39.3 to about 60.7 mg/ml of fibrinogen.

18. A kit for enhancing reperfusion in a patient having a peripheral vascular ischemia comprising fibrinogen, thrombin and an isolated homogenous population of cells obtained from human umbilical cord tissue in an amount effective to treat the disease, wherein the umbilical cord tissue is substantially free of blood, and wherein said isolated homogenous population of the cells is capable of self-renewal and expansion in culture, has the potential to differentiate and do not express CD117 and/or telomerase.

19. The kit of claim 18 further comprising instructions for use.

20. The kit of claims 18 or 19, wherein fibrinogen and the isolated homogenous population of cells are in a composition.

21. The kit of claim 20, wherein thrombin is added to the composition immediately prior to use.

22. The kit of claim 18, wherein the kit comprises from about 16 to about 24 IU/ml of thrombin and from about 39.3 to about 60.7 mg/ml of fibrinogen.

23. The kit of any of claims 18 to 22, wherein the isolated population of cells has one or more of the following characteristics:

(a) expresses oxidized low density lipoprotein receptor 1, reticulon, chemokine receptor ligand 3, and/or granulocyte chemotactic protein;

(b) does not express CD31, CD34 or CD45;

(c) expresses, relative to a human fibroblast, mesenchymal stem cell, or iliac crest bone marrow cell, increased levels of interleukin 8 or reticulon 1;

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