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(54) Title: PROCUREMENT OF PLACENTAL STEM CELLS AND UMBILICAL CORD SEGMENTS

(57) Abstract: Methods, systems, and kits for the procurement of placental stem cells, comprising: a primary cord blood collection; introduction of a cord blood collection solution to the placenta, the cord blood collection solution comprising placental preservative base, a stem cell releasing agent, an antibiotic, and a vasodilator; transporting the primary cord blood collection and the re-filled placenta to a collection/processing center; performing a secondary collection; and storing the collections.

TITLE

PROCUREMENT OF PLACENTAL STEM CELLS AND UMBILICAL CORD SEGMENTS

BACKGROUND

[0001] The present invention relates to placental stem cells and, more specifically, to a method for the procurement of placental stem cells.

2. DESCRIPTION OF THE RELATED ART

[0002] Stem cells are master cells found in all multicellular organisms. These special cells are important to the human body, for example, because they are capable of: (i) differentiating into a multitude of different specialized cell types; and (ii) dividing to maintain a supply of stem cells. In humans there are two main types of stems cells: embryonic stem cells and adult stem cells. In a developing embryo stem cells differentiate into all types of cells, thereby creating specialized tissues, organs, and systems. In an adult human, stem cells are involved in the normal turnover of organs such as blood and skin.

[0003] Hematopoietic stem cells, for example, are used to treat blood and immune system diseases because they can differentiate into red blood cells, white blood cells, and platelets. However, some stem cell transplants have been performed for patients with genetic or metabolic diseases. Indeed, to date more than 80 different diseases have been treated using stem cell transplants. According to the National Cord Blood Program, there were over 15,000 through the end of 2009. The National Marrow Donor Program estimates that there will be 10,000 cord blood transplants per year by 2015, up from 2,000 per year in 2006.

[0004] In addition to known treatments involving stem cells, research continues into the promise of many potential future applications. Indeed, the ability of stem cells to differentiate into other types of cells holds significant promise for treating some of the world's most common diseases including heart disease, diabetes, stroke, hearing loss, blood disorders, Parkinson's disease, and Alzheimer's disease, just to name a few.

[0005] Umbilical cord blood – blood which remains in the placenta and umbilical cord after childbirth – is one of the most common sources of stem cells. Since cord blood is collected from the placenta, which is normally discarded, the collection process is safe for both the mother and the newborn.

[0006] Cord blood is obtained by syringing out the placenta through the umbilical cord shortly after childbirth, after the cord has been detached from the newborn. The retrieved blood can then be frozen and stored indefinitely.

[0007] Although the amount of stem cells obtained from cord blood is generally enough to treat a child, there are generally not enough stem cells to treat an adult patient. The placenta is a better source of stem cells, since it can contain up to ten times more stem cells than cord blood. Still, even when blood is retrieved from both the umbilical cord and placenta using current collection methods, the amount of stem cells is often not suitable to treat an adult patient.

[0008] As a result, there is a continued need for cord blood collection methods and devices that significantly increase the number of stem cells collected, facilitate the collection of stem cells, or allow for the collection of enough stem cells to treat at least one adult patient.

BRIEF SUMMARY

[0009] It is therefore a principal object and advantage to provide a method, device, and/or system for the collection of cord blood.

[0010] It is another object and advantage to provide a method, device, and/or system to increase the number of stem cells collected from cord blood.

[0011] Other objects and advantages will in part be obvious, and in part appear hereinafter.

[0012] According to one embodiment, a method for stem cell collection comprising: (i) providing a placenta comprising cord blood stem cells; (ii) exsanguinating, at least partially, the isolated placenta, wherein the exsanguinated blood is collected; and (iii) perfusing the exsanguinated with a collection solution to form a perfusate comprising cord blood stem cells; wherein the collection solution comprises: (a) a placental preservative base; (b) a stem cell releasing agent; (c) an antibiotic; and (d) a vasodilator.

[0013] According to an aspect, the stem cell releasing agent comprises 1,1′-[1,4-Phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] (also known as AMD3100), which can be present at a concentration of approximately 3 to 10 mg per 100 ml.

[0014] According to another aspect, the antibiotic is an aminoglycoside such as gentamicin, and can be present at a concentration of approximately 10 mg per 100 ml.

[0015] According to an aspect, the vasodilator is one or more of papaverine nitroglycerine, a Ca^{2+} channel blocker, an opium alkaloid, and combinations thereof. The vasodilator can be present at a concentration of approximately 100 mg per 100 ml.

[0016] According to another aspect, the placental preservative base comprises: (i) NaCl; (ii) KCl; (iii) D-Glucose; (iv) citric acid; (v) adenine; (vi) histidine; (vii) glutamate; (viii) glutathione; and (ix) *N*-acetyl-*L*-cysteine. The placental preservative base can

comprise, for example, the following: (i) NaCl at a concentration of approximately 4.3 g/L; (ii) KCl at a concentration of approximately 0.45 g/L; (iii) D-Glucose at a concentration of approximately 1 g/L; (iv) citric acid at a concentration of approximately 2.2 g/L; (v) adenine at a concentration of approximately 0.25 g/L; (vi) histidine at a concentration of approximately 4.2 g/L; (vii) glutamate at a concentration of approximately 1.9 g/L; (viii) glutathione at a concentration of approximately 0.92 g/L or 3 mM; and (ix) *N*-acetyl-*L*-cysteine at a concentration of approximately 0.016 g/L.

[0017] According to an aspect, the collection solution further comprises a synthetic colloidal material, preferably creating colloidal osmotic pressure. The synthetic colloidal material can comprise a hydroxyethyl starch.

[0018] According to another aspect, the collection solution further comprises an anticoagulant such as heparin.

[0019] According to an aspect, the collection solution further comprises a carbohydrate such as trehalose.

[0020] According to another aspect, the method comprises one or more of the additional steps in no particular order: collecting the perfusate; transporting the perfused placenta; isolating the stem cells from the perfusate; and/or combining the collected exsanguinated blood and the collected perfusate.

[0021] According to an aspect, a stem cell collection solution comprising: (i) a placental preservative base; (ii) a stem cell releasing agent; (iii) an antibiotic; and (iv) a vasodilator. The stem cell collection solution can comprise, for example, the following: 1,1′-[1,4-Phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] (AMD3100); gentamicin; and/or papaverine. The placental preservative base can comprises, for example, the following: (i) NaCl; (ii) KCl; (iii) D-Glucose; (iv) citric acid; (v) adenine; (vi) histidine; (vii) glutamate; (viii) glutathione; and/or (ix) *N*-acetyl-*L*-cysteine. The stem cell collection solution can further comprises an anticoagulant.

[0022] According to another aspect, a stem cell collection kit comprising a stem cell collection solution which comprises: a placental preservative base; (ii) a stem cell releasing agent; (iii) an antibiotic; and (iv) a vasodilator.

[0023] According to an aspect of the stem cell collection kit, the stem cell releasing agent comprises 1,1′-[1,4-Phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] (AMD3100), the antibiotic comprises gentamicin, the vasodilator comprises papaverine.

[0024] According to another aspect of the stem cell collection kit, the placental preservative base comprises: (i) NaCl; (ii) KCl; (iii) D-Glucose; (iv) citric acid; (v) adenine; (vi) histidine; (vii) glutamate; (viii) glutathione; and/or (ix) *N*-acetyl-*L*-cysteine.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

[0025] The invention will be more fully understood and appreciated by reading the following Detailed Description in conjunction with the accompanying drawings, in which:

[0026] FIG. 1 is a flowchart of a method of stem cell collection according to one embodiment; and

[0027] FIG. 2 is a flowchart of a method of umbilical cord segment collection according to one embodiment.

DETAILED DESCRIPTION

[0028] Referring now to the drawings, wherein like reference numerals refer to like parts throughout, there is seen in Figure 1 a flowchart of a method of stem cell collection according to one embodiment of the present invention. At step 10 is the primary cord blood collection procedure. This is accomplished using any of a variety of known cord blood collection methods, or any method capable of collecting the excess cord blood from the umbilical cord and/or placenta shortly after childbirth. For example, the cord blood can be collected using the following procedure: (i) deliver the baby vaginally or via cesarean section; (ii) clamp the umbilical cord at both the mother's and the child's side and cut the cord at a location between the clamps; (iii) insert a tube or needle into the umbilical vein to collect the blood via gravity; and (iv) ship the blood off for processing and dispose of the placenta. In a preferred embodiment, the cord blood is collected after delivery of the baby(ies) but before delivery of the placenta.

[0029] As described in the above cord blood collection method, the placenta is typically disposed and/or destroyed upon completion of the typical cord blood collection procedure. As such, these methods do not proceed past step 10 of the present method.

[0030] At step 12 of the present method, the placenta is filled with the cord blood collection solution. In a preferred embodiment, the cord blood collection solution comprises one or more of the following solutions.

[0031] According to one embodiment, the cord blood collection solution comprises the following: (i) placental preservative base; (ii) a stem cell releasing agent; (iii) an antibiotic; and (iv) a vasodilator.

[0032] According to another embodiment, the cord blood collection solution comprises the following: (i) placental preservative base; (ii) a stem cell releasing agent; (iii) an antibiotic; (iv) vasodilator; and (v) synthetic colloidal material.

[0033] According to yet another embodiment, the cord blood collection solution comprises the following: (i) placental preservative base; (ii) a stem cell releasing agent; (iii) an antibiotic; (iv) a vasodilator; (v) a synthetic colloidal material; and (vi) an anticoagulant.

[0034] According to a further embodiment, the cord blood collection solution comprises the following: (i) placental preservative base; (ii) a stem cell releasing agent; (iii) an antibiotic; (iv) a vasodilator; (v) a synthetic colloidal material; and (vi) a carbohydrate.

[0035] According to one embodiment of the present invention, the stem cell releasing agent can be any agent capable of causing stem cells to release from normal storage sites into the blood. According to one embodiment of the present invention, the stem cell releasing agent is AMD3100 (1,1′-[1,4-Phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane], also known by the recommended International Nonproprietary Name plexifor, by the trade name MOZOBIL®, and as JM 3100), preferably at a concentration of approximately 3-10 mg per 100 ml, although other concentrations are possible. The AMD3100 causes, among other conditions, the release of stem cells from the placenta blood vessel walls, resulting in substantially higher amounts of stem cells to be collected via the process described herein.

[0036] The antibiotic can be, for example, any agent capable of providing bactericidal properties against a range of human bacterial infections, while being substantially non-toxic to humans. One such antibiotic is Gentamicin, which is preferably at a concentration of approximately 10 mg per 100 ml in this embodiment, although other concentrations are possible.

[0037] The vasodilator can be any agent capable of dilating the blood vessels of the umbilical cord and placenta. One such vasodilator is papaverine, which is preferably at a concentration of approximately 100 mg per 100 ml in this embodiment, although other concentrations are possible. Other vasodilators include but are not limited to nitroglycerine, Ca^{2+} channel blockers, and other opium alkaloids, for example.

[0038] The synthetic colloidal material can be any synthetic colloidal material, including those intended to create colloidal osmotic pressure and prevent the expansion of the extracellular space in tissues. For example, the synthetic colloidal material can include dextrans, hydroxyethyl starch ("HES") such as Hespan®, and polyethylene glycol. In a preferred embodiment, the synthetic colloidal material is Hespan at a concentration of

approximately 4 to 8%, and preferably 6% (6g/100ml), although other concentrations are possible.

[0039] The anticoagulant can be any compound capable of preventing the coagulation of blood and associated or similar fluids. In a preferred embodiment, the anticoagulant is the sulfated glycosaminoglycan heparin at a concentration of approximately 400 – 600 units per 200 ml, although other concentrations are possible.

[0040] The carbohydrate can be any known natural or synthetic carbohydrate. In a preferred embodiment, the carbohydrate is the disaccharide trehalose at a concentration of 2-6%, and preferably at 4% (4g/100ml), although other concentrations are possible.

[0041] The placental preservative base can be any solution capable of preserving the placenta during shipping and short- and/or long-term storage. According to one embodiment of the present invention, the placental preservative base comprises the following: (i) NaCl (4.3 g/L or 74 mM); (ii) KCl (0.45 g/L or 6 mM); (iii) D-Glucose (1 g/L or 5.6 mM); (iv) citric acid anhydrous (2.2 g/L or 15 mM); (v) adenine free base (0.25 g/L or 1.85 mM); (vi) histidine (4.2 g/L or 20 mM); (vii) glutamate (1.9 g/L or 13 mM); (viii) glutathione (0.92 g/L or 3 mM; and/or (ix) N-Acetyl L-Cysteine (also known as acetylcysteine or *N*-acetylcysteine) (0.016 g/L or 0.1 mM).

[0042] Although a preferred concentration is provided, one of ordinary skill in the art would recognize that one or more of the above concentrations can be varied without affecting the effectiveness or activity of the placental preservative base. Further, one of ordinary skill in the art would recognize that one or more of the above components can be omitted or replaced depending on a variety of factors. According to one embodiment, the target electrolyte and glucose profile of the placental preservative base comprises: (i) approximately 0 to 250 mM Na^+, with a preferred embodiment comprising approximately 125 to 150 mM Na^+; (ii) approximately 0 to 25 mM K^+, with a preferred embodiment comprising approximately 5 to 7 mM K^+; (iii) approximately 0 to 250 mM Cl^-, with a preferred embodiment comprising approximately 50 to 150 mM Cl^-; (iv) 0 to 10 mM glucose, with a preferred embodiment comprising approximately 5 mM glucose; (v) and osmolarity of 100 to 400 mOsm/kg, with a preferred embodiment comprising approximately 280 to 300 mOsm/kg.

[0043] In a preferred embodiment, the cord blood collection solution is allowed to fill the placenta via gravity through a needle or tube inserted into the umbilical vein and/or other locations in the placenta. Once the placenta contains a sufficient amount of cord blood collection solution, decided by a variety of factors including the predetermined amount of solution provided for collection, the size of the placenta, and/or prescribed collection

procedure, among many others, the umbilical cord is once again clamped shut such that the injected solution remains inside the placenta.

[0044] Once the placenta has been refilled with the cord blood collection solution at step 12, the placenta is processed for shipping to a processing/collection center. This can include, among other things, processing the umbilical cord to ensure clamping, placing the placenta in a container suitable for short- or long-term shipping or storage, and/or freezing the placenta. Together with the primary cord blood collection, the placenta is then shipped or delivered to the processing/collection center. At some locations, the processing/collection center may be the same institution or near the same institution as the baby delivery center.

[0045] At step 16, the placenta undergoes processing at the processing/collection center. The cord blood collection solution is collected from the placenta at this step. This "secondary collection" is then processed to concentrate the mononuclear cell content and is then cryogenically stored together, but in separate containers, with the primary cord collection. The two collections may or may not be combined prior to storage depending upon a variety of factors, including but not limited to storage limitations and known or possible future uses of the stem cells, among others. The placenta can be cryogenically stored as well, or can be disposed of once the procedure is complete. In a preferred embodiment, the cord blood collection solution is allowed to drain from the placenta via gravity through a needle or tube inserted into the umbilical vein and/or other locations in the placenta. To begin draining, the umbilical cord is unclamped to allow the egress of fluid, and any drained fluid is then collected.

[0046] In yet another embodiment, the placenta is cryogenically stored prior to the secondary collection. In this embodiment, the injected cord blood collection solution is stored inside the placenta until it is needed, or until it can be more properly processed.

[0047] Referring now to Figure 2, there is seen a flowchart of a method of umbilical cord segment collection according to one embodiment of the present invention. Umbilical cord segment collection is a growing field, as these segments contain materials that can be cultivated and manipulated for beneficial uses. For example, stem cells can be harvested from the segment and used to grow a sufficiently large collection of stem or differentiated (fully or partially) cells which themselves can be used immediately or stored for future possible use.

[0048] At step 20 of the method is the collection and cleaning of the umbilical cord. Following delivery of the baby vaginally or via cesarean section, the umbilical cord is harvested and can be cleaned using any suitable technique for sterilizing the exterior surface

of the cord to prevent contamination. Following cleaning, at step 22, a segment of the umbilical cord of a desired length is cut out. For example, the desired length may be a 1-inch long segment of the cord, or may be longer or shorter depending upon the downstream processing needs. In a preferred embodiment, the umbilical cord is cut using sterile tools and techniques. At step 24 of the method, the cut segment is placed into the umbilical cord storage/collection solution. The solution can be any of the previous solutions known in the art or any of the novel solutions described herein. In a preferred embodiment, the collector has a tube pre-filled with the solution and simply places the umbilical cord segment into the tube (thereby immersing it in the solution), perhaps with mixing by shaking or inverting the tube several times. In a preferred embodiment the tube is sized to fit the exact size of the umbilical cord segment and the solution. Once in the solution and in the tube, the umbilical cord segment is secure and the cells and tissues within that umbilical cord segment are well-maintained.

[0049] Once immersed in the solution, the umbilical cord segment is ready for shipment or deliver to a collection or storage center at step 26 of the method. The segment can be immediately processed at the center, or can be stored for processing in the future.

[0050] According to another aspect is a system for the procurement of placental stem cells and/or umbilical cord segments. The system may comprise all of some of the components needed to procure cells from a placenta and/or collect umbilical cord segments. For example, the system may comprise a device for placental harvesting and stabilization during processing of the placenta according to one or more of the methods disclosed herein. The system may also comprise, for example, clamps, needles, tubing, scissors, scalpel, or other components to facilitate the procurement of cells from a placenta. In addition, many other devices known in the art for harvesting, stabilizing, holding, and/or transporting a placenta would be suitable for the system.

[0051] The system may also comprise a transportation element, such as a sleeve, container, envelope, or other element comprising space for transportation of the placenta to a collection and/or storage site. The transport element may also comprise space for transporting all of some of the collected liquid/cells from the placenta to the collection and/or storage site.

[0052] The system comprises cord blood collection solution, according to an embodiment. The components may be stored as a single solution, or one or more of the components may be stored separately and require mixing with the remainder of the solution prior to collection. According to one embodiment, and as described in further detail

elsewhere herein, the cord blood collection solution comprises a container, bag, or other holding element containing the following: (i) placental preservative base; (ii) a stem cell releasing agent; (iii) an antibiotic; and (iv) a vasodilator. The cord blood collection solution may also comprise a synthetic colloidal material, an anticoagulant, and/or a carbohydrate. According to one embodiment, the placental preservative base comprises one or more of the following: NaCl, KCl, D-Glucose, citric acid anhydrous, adenine free base, histidine, glutamate, glutathione, and/or N-Acetyl L-Cysteine.

[0053] According to yet another aspect is a kit for the procurement of placental stem cells and/or umbilical cord segments. The kit may comprise all of some of the components needed to procure cells from a placenta and/or collect umbilical cord segments. For example, the kit may comprise a device for placental harvesting and stabilization during processing of the placenta according to one or more of the methods disclosed herein. The kit may also comprise, for example, clamps, needles, tubing, scissors, scalpel, or other components to facilitate the procurement of cells from a placenta and/or the collection of umbilical cord segments. In addition, many other devices known in the art for harvesting, stabilizing, holding, and/or transporting a placenta and/or collect umbilical cord segments would be suitable for the kit.

[0054] The kit may also comprise a transportation element, such as a sleeve, container, envelope, or other element comprising space for transportation of the placenta to a collection and/or storage site. The transport element may also comprise space for transporting all of some of the collected liquid/cells from the placenta to the collection and/or storage site.

[0055] The kit comprises cord blood collection solution, according to an embodiment. The components of the solution may be stored as a single solution, or one or more of the components may be stored separately and require mixing with the remainder of the solution prior to collection. According to one embodiment, and as described in further detail elsewhere herein, the cord blood collection solution comprises a container, bag, or other holding element containing the following: (i) placental preservative base; (ii) a stem cell releasing agent; (iii) an antibiotic; and (iv) a vasodilator. The cord blood collection solution may also comprise a synthetic colloidal material, an anticoagulant, and/or a carbohydrate. According to one embodiment, the placental preservative base comprises one or more of the following: NaCl, KCl, D-Glucose, citric acid anhydrous, adenine free base, histidine, glutamate, glutathione, and/or N-Acetyl L-Cysteine.

[0056] Although the present invention has been described in connection with a preferred embodiment, it should be understood that modifications, alterations, and additions can be made to the invention without departing from the scope of the invention as defined by the claims.

CLAIMS

What is claimed is:

1. A method for stem cell collection, the method comprising:

providing a placenta comprising cord blood stem cells;

exsanguinating, at least partially, the isolated placenta, wherein the exsanguinated blood is collected; and

perfusing the exsanguinated with a collection solution to form a perfusate comprising cord blood stem cells;

wherein the collection solution comprises: (i) a placental preservative base; (ii) a stem cell releasing agent; (iii) an antibiotic; and (iv) a vasodilator.

2. The method of claim 1, wherein the stem cell releasing agent comprises 1,1′-[1,4-Phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] (AMD3100).

3. The method of claim 1, wherein the concentration of the stem cell releasing agent in the collection solution is approximately 3 to 10 mg per 100 ml.

4. The method of claim 1, wherein the antibiotic is an aminoglycoside.

5. The method of claim 4, wherein the antibiotic is gentamicin.

6. The method of claim 1, wherein the concentration of the antibiotic in the collection solution is approximately 10 mg per 100 ml.

7. The method of claim 1, wherein the vasodilator is selected from the group consisting of nitroglycerine, a Ca^{2+} channel blocker, an opium alkaloid, and combinations thereof.

8. The method of claim 1, wherein the vasodilator comprises papaverine.

9. The method of claim 1, wherein the concentration of the vasodilator in the collection solution is approximately 100 mg per 100 ml.

10. The method of claim 1, wherein the placental preservative base comprises: (i) NaCl; (ii) KCl; (iii) D-Glucose; (iv) citric acid; (v) adenine; (vi) histidine; (vii) glutamate; (viii) glutathione; and (ix) *N*-acetyl-*L*-cysteine.

11. The method of claim 1, wherein the placental preservative base comprises: (i) NaCl at a concentration of approximately 4.3 g/L; (ii) KCl at a concentration of approximately 0.45 g/L; (iii) D-Glucose at a concentration of approximately 1 g/L; (iv) citric acid at a concentration of approximately 2.2 g/L; (v) adenine at a concentration of approximately 0.25 g/L; (vi) histidine at a concentration of approximately 4.2 g/L; (vii) glutamate at a concentration of approximately 1.9 g/L; (viii) glutathione at a concentration of

approximately 0.92 g/L or 3 mM; and (ix) *N*-acetyl-*L*-cysteine at a concentration of approximately 0.016 g/L.

12. The method of claim 1, wherein the collection solution further comprises a synthetic colloidal material.

13. The method of claim 12, wherein the synthetic colloidal material creates colloidal osmotic pressure.

14. The method of claim 12, wherein the synthetic colloidal material comprises a hydroxyethyl starch.

15. The method of claim 1, wherein the collection solution further comprises an anticoagulant.

16. The method of claim 15, wherein the anticoagulant comprises heparin.

17. The method of claim 1, wherein the collection solution further comprises a carbohydrate.

18. The method of claim 17, wherein the carbohydrate comprises trehalose.

19. The method of claim 1, further comprising the step of collecting the perfusate.

20. The method of claim 1, further comprising the step of transporting the perfused placenta.

21. The method of claim 1, further comprising the step of isolating the stem cells from the perfusate.

22. The method of claim 1, further comprising the step of combining the collected exsanguinated blood and the collected perfusate.

23. A stem cell collection solution comprising: (i) a placental preservative base; (ii) a stem cell releasing agent; (iii) an antibiotic; and (iv) a vasodilator.

24. The stem cell collection solution of claim 23, wherein said stem cell releasing agent comprises 1,1′-[1,4-Phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] (AMD3100), said antibiotic comprises gentamicin, said vasodilator comprises papaverine.

25. The stem cell collection solution of claim 23, wherein said placental preservative base comprises: (i) NaCl; (ii) KCl; (iii) D-Glucose; (iv) citric acid; (v) adenine; (vi) histidine; (vii) glutamate; (viii) glutathione; and (ix) *N*-acetyl-*L*-cysteine.

26. The stem cell collection solution of claim 23, further comprising an anticoagulant.

27. A stem cell collection kit, the kit comprising a stem cell collection solution, the stem cell collection solution comprising: a placental preservative base; (ii) a stem cell releasing agent; (iii) an antibiotic; and (iv) a vasodilator.

28. The stem cell collection kit of claim 27, wherein said stem cell releasing agent comprises 1,1′-[1,4-Phenylenebis(methylene)]bis[1,4,8,11-tetraazacyclotetradecane] (AMD3100), said antibiotic comprises gentamicin, said vasodilator comprises papaverine.

29. The stem cell collection kit of claim 27, wherein said placental preservative base comprises: (i) NaCl; (ii) KCl; (iii) D-Glucose; (iv) citric acid; (v) adenine; (vi) histidine; (vii) glutamate; (viii) glutathione; and (ix) *N*-acetyl-*L*-cysteine.

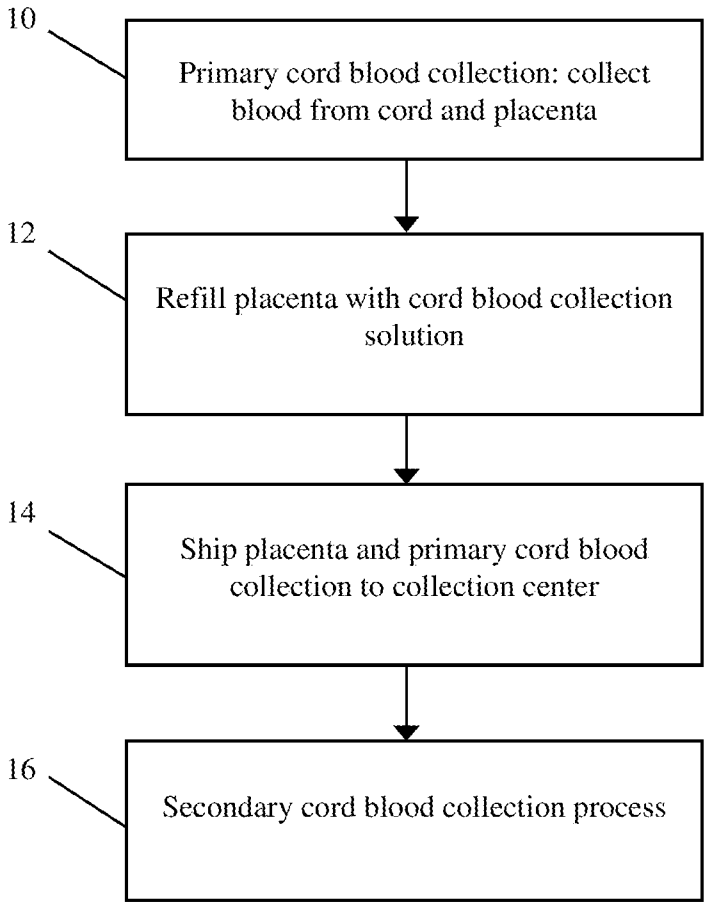

10
Primary cord blood collection: collect blood from cord and placenta
12
Refill placenta with cord blood collection solution
14
Ship placenta and primary cord blood collection to collection center
16
Secondary cord blood collection process

FIG. 1

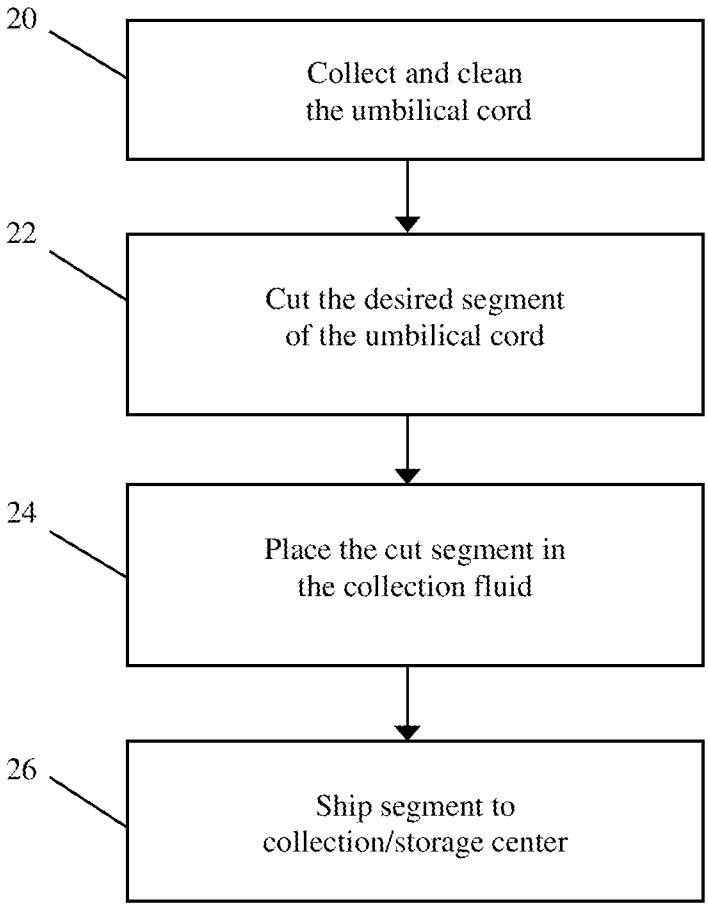

20
Collect and clean the umbilical cord
22
Cut the desired segment of the umbilical cord
24
Place the cut segment in the collection fluid
26
Ship segment to collection/storage center

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/023868

A. CLASSIFICATION OF SUBJECT MATTER

C12N 5/073(2010.01)i, C12N 5/02(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N 5/073; A01N 1/02; C12N 5/00; C12N 5/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & keywords: stem cell, placenta, cord blood, preservative base, stem cell releasing agent, antibiotic, vasodilator

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010-0291679 A1 (EDINGER, JAMES W. et al.) 18 November 2010 See paragraphs [0010], [0259]-[0260], [0273], [0537]-[0538], [0554]-[0555] and [0572]; claims 36, 43, 47-48, 51 and 53-54.	1-3,6,7,9,11-16,19-23,26,27
A		4,5,8,10,17,18,24,25,28,29
A	US 2007-0190649 A1 (GAGE, FRED) 16 August 2007 See paragraphs.[0031], [0035], [0040] and [0045]-[0046], claims 1, 3-4 and 12-14.	1-29
A	US 2010-0248206 A1 (KUYPERS, FRANS A. et al.) 30 September 2010 See paragraphs [0033] and [0037]; claims 1, 4 and 7.	1-29
A	US 2010-0323446 A1 (BARNETT, JILL RENEE et al.) 23 December 2010 See paragraphs [0037]-[0038], [0040], [0051], [0113] and [0120]; claims 1-2 and 19-20.	1-29
A	TSAGIAS, NIKOS et al., `Placenta perfusion has hematopoietic and mesenchymal progenitor stem cell potential`, Transfusion, May 2011, Vol. 51, No. 5, pp. 976-985. See page 977.	1-29

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 June 2013 (20.06.2013)	**21 June 2013 (21.06.2013)**

Name and mailing address of the ISA/KR
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2013/023868

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