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	This document serves as an Oath/Declaration (37 CFR 1.63).

Total Attachments: 46
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Systems and methods for deriving, preparing and administering adult mesenchymal autologous stem cells

Abstract

The invention provides a system and methodology for deriving, preparing, and administering platelet rich plasma and adult mesenchymal autologous stem cells suitable for regenerating tissue in a living organism. The system includes a sealed primary container capable of removing blood from a living organism. The system further includes a sealed secondary container containing a separation medium and a low-density high-viscosity liquid for receiving the blood. The system further includes a collecting device with a housing and a member wherein the member is moveable in a distal direction is useful in collecting and/or dispensing medium. The system further includes a separation medium capable of separating red blood cells from plasma when the container contains blood and is centrifuged. The system further includes a sealed or unsealed container with a transfer device designed to remove the platelet rich plasma and the adult mesecnhymal autologous stem cells from the blood. The system also includes a transfer device having a apparatus adapted to deliver and/or puncture a fourth container in order to provide fluid communication between the third and fourth containers. The system also includes a transfer device having an apparatus to deliver the adult mesenchymal autologous stem cells to the living organism.

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Field of Search: 604/403-416,8-10,57 210/650,651,781,782,787 422/101,102

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Claims

We claim:

1.A system for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells suitable for regenerating tissue in a living organism; the system comprising a sealed primary container having a single opening configured and capable of removing blood from a living organism; the system further including a sealed secondary container containing a separation medium mixed with the receiving blood. The system further includes a collecting device with a housing and a member wherein the member is moveable in a distal direction and is useful in collecting and/or dispensing medium. The system further includes a separation medium capable of separating red blood cells from platelet rich plasma and adult mesenchymal autologous stem cells when the container contains blood and is centrifuged; a sealed or unsealed tertiary container with a transfer device designed to remove the platelet rich plasma and the adult mesecnhymal autologous stem cells from the blood; a transfer device having a apparatus adapted to deliver and/or puncture a fourth container in order to provide fluid communication between the third and fourth containers. The system also includes a transfer device having an apparatus to deliver the platelet rich plasma and adult mesenchymal autologous stem cells to the living organism.

2. The system of claim 1, wherein the primary container removes blood from the living organism and is delivered into a secondary container.

3. The system of claim 1, wherein the secondary container contains a separation medium of ionic coagulation activator is selected from but not limited to; heparin, bivalirudin, a similar derivative and/or combinations thereof.

4. The system of claim 1, wherein the system includes a collecting device with a housing and a member wherein the member is moveable in a distal direction and is useful in collecting and/or dispensing medium.

5. The system of claim 1, wherein a separation medium in the secondary container is capable of separating red blood cells from platelet rich plasma and adult mesenchymal stem cells when the container contains blood and is centrifuged.

6. The system of claim 1, wherein a sealed and/or unsealed container with a transfer device designed to remove the platelet rich plasma and the adult mesenchymal autologous stem cells from the secondary container leaving only the blood.

7. The system of claim 1, wherein a transfer device having an apparatus adapted to deliver and/or puncture a fourth container in order to provide fluid communication between the third and fourth containers.

8. The system of claim 1, wherein a transfer device having an apparatus to deliver the platelet rich plasma and adult mesenchymal autologous stem cells to the living organism.

9. A system for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells for regenerating tissue in a living organism. The system comprising: a primary container capable of removing blood from a living organism, a sealed secondary container containing a separation medium mixed with the receiving blood, a collecting device with a housing and a member wherein the member is moveable in a distal direction is useful in collecting and/or dispensing medium, a separation medium capable of separating red blood cells from platelet rich plasma and adult mesenchymal stem cells when the container contains blood and is centrifuged, a sealed or unsealed container with a transfer device designed to remove the platelet rich plasma and the adult mesenchymal autologous stem cells from the secondary container leaving only the blood, a transfer device having a apparatus adapted to deliver and/or puncture a fourth container in order to provide fluid communication between the third and fourth containers, and a transfer device having an apparatus to deliver the platelet rich plasma and adult mesenchymal autologous stem cells to the living organism.

10. The system of claim 9, wherein the primary container is sealed and capable of removing blood from a living organism.

11. The system of claim 9, wherein the separation medium of ionic coagulation activator is selected from, but not limited to; heparin, bivalirudin, a similar derivative and/or combinations thereof.

12. The system of claim 9, wherein the secondary container further contains an anti-coagulant.

13. The system of claim 9, wherein the system includes a collecting device with a housing and a member wherein the member is moveable in a distal direction is useful in collecting and/or dispensing medium.

14. The system of claim 9, wherein a separation medium capable of separating red blood cells from platelet rich plasma and adult mesenchymal autologous stem cells when the container contains blood and is centrifuged.

15. The system of claim 9, wherein a sealed and/or unsealed container with a transfer device designed to remove the platelet rich plasma and the adult mesenchymal autologous stem cells from the blood.

16. The system of claim 9, wherein a transfer device having an apparatus adapted to deliver and/or puncture a fourth container in order to provide fluid communication between the third and fourth containers.

17. The system of claim 9, wherein a transfer device having an apparatus to deliver the platelet rich plasma and adult mesenchymal autologous stem cells to the living organism.

18. A system for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells suitable for regenerating tissue in a living organism, the system comprising: a primary container having a single opening configured to remove blood from a living organism; a secondary container capable of receiving blood and is capable to receive a cap to form a seal therewith, the cap capable of being removed from the container and/or pierced in sealing the container for delivery of blood into the secondary container, the secondary container containing a separation medium capable of separating red blood cells from platelet rich plasma and adult mesenchymal stem cells when the container is centrifuged; a collecting device with a housing and a member wherein the member is moveable in a distal direction is useful in collecting and/or dispensing medium, a sealed or unsealed container with a transfer device designed to remove the platelet rich plasma and the adult mesenchymal autologous stem cells from the blood, the device configured to pierce the cap or in lieu of removing the cap, transfer a portion of the platelet rich plasma and adult mesenchymal autologous stem cells to a device; the device configured to pierce the cap and transfer a portion of the platelet rich plasma and the adult mesenchymal autologous stem cells from the secondary container to the third container via pressure differentiation upon establishment of fluid communication between the cannula of the third container undergoing removal of the platelet rich plasma and the adult mesenchymal autologous stem cells until only the portion of the red blood cells remains in the secondary container.

19. The system of claim 18, wherein the device comprises a cannula attached to a collecting device with a housing and a member wherein the member is moveable in a distal direction is useful in collecting and/or dispensing medium, a separation device capable of separating platlet rich plasma and adult mesenchymal autologous stem cells from red blood cells after the container has been centrifuged to separate the platelet rich plasma and the adult mesenchymal autologous stem cells until only the portion of the red blood cells remain in the secondary container.

20. The system of claim 18, wherein a transfer device having a apparatus adapted to deliver and/or puncture a fourth container in order to provide fluid communication between the third and fourth containers.

21. The system of claim 18, wherein the third container further includes a transfer device having an apparatus to deliver the platelet rich plasma and adult mesenchymal autologous stem cells to the living organism.

Description

This patent application fully incorporates by reference the subject matter of each of the above-identified patent applications to which this application claims priority. The entire disclosure of each patent application is considered to be part of the accompanying application.

BACKGROUND OF THE INVENTION

The present invention relates to a system and methodology for deriving, preparing and administering platlet rich plasma and adult mesenchymal autologous stem cells suitable for regenerating tissue in a living organism.

Adult mesenchymal autologous stem cells are known to divide or self-renew indefinitely and their differentiation potential is unknown and unlimited. Adult mesenchymal autologous stem cell treatments have been successfully used for many years to treat leukemia and related bone/blood cancers through bone marrow transplants.

Several kits are available on the market that contain adult mesenchymal autologous stem cells from donors.

Such known kits involve the use of material of human or animal origin, which, owing to its origin, could result in possible viral contamination and in serious risks for the receiver of the donored adult mesenchymal autologous stem cells. In the past the authorities have been compelled to suspend from trade or even ban the stem cell derivatives obtained by using material of human or animal origin. Furthermore, rejection cases are known from the literature resulting from reimplanting donored adult mesenchymal autologous stem cells produced by using human or animal proteins in patients. Such cases are indeed due to the donored origin, with respect to the receiver organism, of the donored protein being reimplanted or some of the components used for preparing it.

The adult mesenchymal autologous stem cells obtained from a patient's own blood, is more reliable with respect to the rejection and/or infection risks. Several procedures have already been described for obtaining extemporary adult mesenchymal autologous stem cells, but no system and methodology for deriving, preparing and administering adult mesenchymal autologous stem cells suitable for regenerating tissue in a living organism is available on the market although some relevant references with similarities can be found in the patent literature.

U.S. Pat. No. 5,733,545 discloses a plasma-buffy coat concentrate to be combined with a fibrinogen activator to form a platelet glue wound sealant. The method disclosed in this patent allows for a patient's blood to be processed in order to obtain autologous fibrin glue, but the methods use thrombin or batroxobin as the fibrinogen activator. These activators are of human or animal nature and therefore still involve the risk of rejection and/or viral infections for the patient.

U.S. Pat. No. 5,555,007 discloses a method and an apparatus for making concentrated plasma to be used as a tissue sealant. The method consists in separating plasma from whole blood and removing water from said plasma by contacting it with a concentrator to provide concentrated

plasma which can be thereafter coagulated with a solution containing thrombin and calcium. The apparatus comprises a first centrifuge separator in a first chamber, a concentrator (e.g. dextranomer or polyacrylamide) included in a second chamber communicating with the first chamber, and a second separator. The method disclosed in this reference requires a long time for obtaining the plasma concentrate necessary for the subsequent preparation of autologous fibrin glue and the apparatus is expensive and not disposable. The method does not disclose using a calcium-coagulation activator, and requires a pre-concentration step.

Overall, methods and systems for deriving, preparing and administering adult mesenchymal autologous stem cells suitable for regenerating tissue in a living organism are desired.

DETAILED DESCRIPTION OF THE DRAWINGS

FIG. 1 is a perspective view of a first embodiment of the invention.

FIG. 2 is a cross-sectional view of a closed container of the first embodiment shown in FIG. 1 wherein the primary container removes blood from the living organism.

FIG. 3 is a cross-sectional view of a secondary container of the first embodiment shown in FIG. 1 wherein the secondary container contains an anti-coagulant and is capable of receiving blood through the closed container of the first embodiment.

FIG. 4 is a cross-sectional view of a second embodiment of the invention showing collecting device with a housing and a member wherein the member is moveable in a distal direction is useful in collecting and/or dispensing medium.

FIG. 5 is a cross-sectional view of a different embodiment of the closed container of the first embodiment shown in FIG. 1 wherein the primary container removes blood from the living organism and is transferred to a secondary container containing an anti-coagulant.

FIG. 6 is a cross-sectional view of the secondary container of the first embodiment of FIG. 3 housed in a container with a separation medium capable of separating red blood cells from plasma when the container contains blood before being centrifuged.

FIG. 7 is the cross-sectional view of a device capable of separating red blood cells from plasma when the container contains blood and is centrifuged.

FIG. 8 is a cross-sectional view of a different embodiment when the secondary container containing blood and a separation medium is placed in a device capable of separating red blood cells from plasma when the container is centrifuged.

FIG. 9 is a cross-sectional view of a different embodiment after the secondary container containing blood and a separation medium has been placed in a device capable of separating red blood cells from plasma when the container is centrifuged depicting the separation of whole blood, adult mesenchymal autologous stem cells and platelet rich plasma.

FIG. 10 is a cross-sectional view of a second embodiment of the invention wherein a sealed or unsealed container with a transfer device designed to remove the platelet rich plasma and the adult mesenchymal autologous stem cells from the blood, the device configured to pierce the cap or in lieu of removing the cap is engaged.

FIG. 11 is a cross-sectional view of FIG. 10 showing the secondary container and transfer device engaged, and the contents of the second container being transferred to the third container of the transfer device leaving only the whole blood in the secondary container.

FIG. 12 is a cross-sectional view of a different embodiment of the transfer device containing the platelet rich plasma and the adult mesenchymal autologous stem cells.

FIG. 13 is a cross-sectional view of the third embodiment of the invention wherein the collecting device with a housing and a member wherein the member is moveable in a distal direction, with its contents, is able to dispense its medium into a second medium housed in a container for multiplying, regenerating, duplicating and incubating adult mesencymal autologous stem cells for future use.

FIG. 14 is a cross-sectional view of the fourth embodiment of the invention wherein the collecting device with a housing and a member wherein the member is moveable in a distal direction, with its contents, is able to dispense its medium into an area that includes but is not limited to; musculoskeletal, ligament, tendon, cartilage, meniscus, and disc of a living organism for regeneration of said area.

FIG. 15 is a cross-sectional view of the fifth embodiment of the invention wherein the collecting device with a housing and a member wherein the member is moveable in a distal direction, with its contents, is able to dispense its medium into an area that includes but is not limited to; musculoskeletal, ligament, tendon, cartilage, meniscus, and disc of a living organism for regeneration of said area.

FIG. 16 is a cross-sectional view of the sixth embodiment of the invention wherein the collecting device with a housing and a member wherein the member is moveable in a distal direction, with its contents, is able to dispense its medium into a fifth container that contains but is not limited to sodium chloride or similar solution.

FIG. 17 is a cross-sectional view of the sixth embodiment of the invention wherein the methodology of dispensing the medium containing the platelet rich plasma and adult mesencymal autologous stem cells into a living organism via intravenous or intra-arterial.

FIG. 18 is a cross-sectional view of the seventh embodiment of the invention wherein the collecting device with a housing and a member wherein the member is moveable in a distal direction, with its contents, is able to dispense its medium into a sixth container that contains but is not limited to albuterol, sodium chloride or similar solution.

FIG. 19 is a cross-sectional view of the seventh embodiment of the invention wherein the methodology of dispensing the medium containing the adult mesencymal autologous stem cells into a living organism via inhalation.

FIG. 20 is a cross-sectional view of a ready-to-use step-by-step system and methodology packaged in such a way that the accoutrements include all of the components required for deriving, preparing and administering adult mesenchymal autologous stem cells suitable for regenerating tissue in a living organism.

Before one embodiment of the invention is explained in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or being carried out in various ways. Also, it is to be understood that the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting.

SUMMARY OF THE INVENTION

In one aspect, the invention provides a system and methodology for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells suitable for regenerating tissue in a living organism. The system comprises a primary container capable of removing blood from a living organism, a sealed secondary container containing a separation medium mixed with the receiving blood. The system further comprises a collecting device with a housing and a member wherein the member is moveable in a distal direction is useful in collecting and/or dispensing medium. The system further has a separation medium capable of separating red blood cells from plasma when the container contains blood and is centrifuged. The system also has a sealed or unsealed container with a transfer device designed to remove the platelet rich plasma and the adult mesenchymal autologous stem cells from the blood. The transfer device has an apparatus adapted to deliver and/or puncture a fourth container in order to provide fluid communication between the third and fourth containers. The system also has a transfer device having an apparatus to deliver the platelet rich plasma and adult mesenchymal autologous stem cells to the living organism.

In another aspect, the invention provides another system for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells suitable for regenerating tissue in a living organism. The system comprises a primary container capable of removing blood from a living organism. The system further comprises a secondary container

capable of receiving blood from the primary container and having a single opening configured to receive a cap to form a seal therein. The system further comprises a cap capable of being removed from the container and/or pierced in sealing the container for delivery of blood into the secondary container. The system also comprises a secondary container containing a separation medium capable of separating red blood cells from plasma when the container is centrifuged. The system further comprises a collecting device with a housing and a member wherein the member is moveable in a distal direction is useful in collecting and/or dispensing medium, a separation medium capable of separating red blood cells from platelet rich plasma and adult mesenchymal autologous stem cells when the container contains blood and is centrifuged. The system also includes a sealed or unsealed container with a transfer device designed to remove the platelet rich plasma and the adult mesenchymal autologous stem cells from the blood. The system also comprises a device configured to pierce the cap or in lieu of removing the cap, transfer a portion of the platelet rich plasma and adult mesenchymal autologous stem cells to a device. The system also includes a device configured to pierce the cap and transfer a portion of the platelet rich plasma and the adult mesenchymal autologous stem cells from the secondary container to the third container via pressure differentiation upon establishment of fluid communication between the cannula of the third container undergoing removal of the platelet rich plasma and the adult mesenchymal autologous stem cells until only the portion of the red blood cells remains in the secondary container.

In another aspect, the invention provides a method for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells suitable for regenerating tissue in a living organism. The method comprises drawing blood from a patient through a primary container into a secondary container containing an anti-coagulant. The blood is transferred from a secondary container. The blood is centrifuged allowing a separation of the platelet rich plasma and the adult mesencymal autologous stem cells from the whole blood. A collecting device with a housing and a member wherein the member is moveable in a distal direction is utilized in collecting the medium. The collecting device is then able to dispense its medium into an area that includes but is not limited to; musculoskeletal, ligament, tendon, cartilage, meniscus, and disc of a living organism for regeneration of said area.

In another aspect, the invention provides a method for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells suitable for regenerating tissue in a living organism. The method comprises drawing blood from a patient through a primary container into a secondary container containing an anti-coagulant. The blood is transferred from a secondary container. The blood is centrifuged allowing a separation of the platelet rich plasma and the adult mesencymal autologous stem cells from the whole blood. A collecting device with a housing and a member wherein the member is moveable in a distal direction is utilized in collecting the medium. The collecting device with a housing and a member wherein the member is moveable in a distal direction, with its contents, is able to dispense its medium into a second

medium housed in a container for multiplying, regenerating, duplicating and incubating adult mesencymal autologous stem cells for future use.

In another aspect, the invention provides a method for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells suitable for regenerating tissue in a living organism. The method comprises drawing blood from a patient through a primary container into a secondary container containing an anti-coagulant. The blood is transferred from a secondary container. The blood is centrifuged allowing a separation of the platelet rich plasma and the adult mesencymal autologous stem cells from the whole blood. A collecting device with a housing and a member wherein the member is moveable in a distal direction is utilized in collecting the medium. The collecting device with a housing and a member wherein the member is moveable in a distal direction, with its contents, is able to dispense its medium into a separate container that contains but is not limited to sodium chloride or similar solution. The container containing the medium of platelet rich plasma, adult mesenchymal autologous stem cells and sodium chloride or similar medium can now be dispensed into a living organism via intravenous or intra-arterial.

In another aspect, the invention provides a method for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells suitable for regenerating tissue in a living organism. The method comprises drawing blood from a patient through a primary container into a secondary container containing an anti-coagulant. The blood is transferred from a secondary container. The blood is centrifuged allowing a separation of the platelet rich plasma and the adult mesencymal autologous stem cells from the whole blood. A collecting device with a housing and a member wherein the member is moveable in a distal direction is utilized in collecting the medium. The collecting device with a housing and a member wherein the member is moveable in a distal direction, with its contents, is able to dispense its medium into a separate container that contains but is not limited to sodium chloride, albuterol, or a similar solution. The container containing the medium of platelet rich plasma, adult mesenchymal autologous stem cells and sodium chloride or similar medium can now be dispensed into a living organism via inhalation.

DETAILED DESCRIPTION OF THE INVENTION

The object of the present invention is therefore to provide a system and methodology for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells suitable for regenerating tissue in a living organism without resulting in viral infections and/or rejection cases when used in surgery.

Such an object is achieved by using platelet rich plasma and adult mesenchymal autologous stem cells obtained from a patient's own blood, is more reliable with respect to the rejection and/or infection risks, with little if any side effects.

Furthermore, rejection cases are known from the literature resulting from reimplanting donored stem cells produced by using human or animal proteins in patients. Such cases are indeed due to the donored origin, with respect to the receiver organism of the donored protein being reimplanted or some of the components used for preparing it.

The system and methodology for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells according to the present invention comprises a sealed container containing an anti-coagulant. The anti-coagulant separates the platelet rich plasma and adult mesenchymal autologous stem cells from the whole blood when it is introduced into the sealed container.

The system and methodology for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells according to the present invention have the great advantage of allowing the preparation of platelet rich plasma and adult mesenchymal autologous stem cells which may be used with no risk of viral infections or rejection cases.

Another advantage of the methodology for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells according to the present invention is that it allows the preparation of platelet rich plasma and adult mesenchymal autologous stem cells from patient's plasma in a very short time as well as in the formation of liquid, membranous coatings, injectables, intravenous, intra-arterial or spray. Still another advantage of the methodology for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells according to the present invention is that it allows the preparation of platelet rich plasma and adult mesenchymal autologous stem cells to be obtained at costs proportionally lower with respect to the known systems.

Further advantages of the methodology for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells to the present invention will be evident to those skilled in the art from the following detailed description of some embodiments thereof.

Containers suitable for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells according to the present invention include a glass container for blood collection as hereinafter described in Example 1. Also glass or plastic test-tubes may be used. The preferred volume of the container is from 5 to 15 ml. with preference being 10 ml. The test-tubes have preferably a diameter ranging from 12 to 16 mm and a height ranging from 75 to 100 mm. with preference being 16mm in diameter and 100mm in height. The container should be suitably thick in order to withstand the stresses resulting from the pressure difference between its inner space and the atmosphere when it is evacuated. Hemispherical or conical bottom tubes are preferably 0.7 mm thick, flat bottom tubes 1 mm thick. The plastic containers are preferably made of transparent polyester resin, 0.2-0.8 mm thick, in order to ensure the vacuum keeping for at least 12 months after production. After the preparation, the plastic test-

tubes, are preferably introduced into a tin-foil vacuum air-tight container having a heat-sealed inner polyethylene layer in order to ensure a perfect air-tightness until the date of use.

It should be noted that the evacuation of containers or test-tubes is advisable, however not necessary for putting the present invention into practice.

The containers or test-tubes are sealed by rubber or silicon pierceable caps, being suitable to ensure the container to be perfectly air-tight and to allow the vacuum plugging before the introduction of the blood and during evacuation of the platelet rich plasma and the adult mesenchymal stem cells step.

EXAMPLES

Example 1

30 ml of venous blood were drawn from a patient according to the provisions of the qualitative standards for clinical analysis, e.g. by using VACUTAINER.RTM. sterile test-tubes by Becton-Dickinson, added with a 0.106 M sodium citrate solution. For this purpose also test-tubes added with disodium or dipotassium ethylenediaminetetraacetate can be used. The sample was carefully kept sterile during the blood drawing. Finally, the sample was gently shaken for wholly mixing the components, thereby ensuring the anticoagulating action of sodium citrate. The test-tube was then introduced in a suitable centrifuge, while carefully balancing the rotor weight in order to prevent the same centrifuge to be damaged. Once the lid is sealed, the sample was centrifuged at 3500 rpm for 15 minutes, thereby separating the red cells (being thicker) from the citrated plasma (supernatant). In this case the plasma yield, mainly depending upon the characteristics of the donor blood, was as high as 65%. The test-tube containing the separated plasma was kept plugged in sterile conditions and was placed vertically in a stand for recovering the plasma itself, in this step care was taken not to shake the test-tube, in order to prevent the mixing of the two phases separated in the centrifugation. The outer portion of the test-tube cap was then sterilized by using denatured alcohol, then carefully removed. A sterile needle, being connected to a sterile syringe, was introduced in the test-tube cap. The needle was brought up to 3-4 mm apart from the separating meniscus of the two phases, and 12 ml of platelet rich plasma were drawn along with the buffy coat containing the adult mesenchymal autologous stem cells. The platelet rich plasma yield was then inserted into (4) second sterile syringes each containing 3 ml of platelet rich plasma and adult mesenchymal autologous stem cells and a sterile needle of choice was connected to each sterile syringe containing the platelet rich plasma and adult mesenchymal autologous stem cells, now ready to be immediately used.

Example 2

Approximately 30 ml of venous blood was drawn from a normotype 19 years-old patient presenting a tear of the left medial meniscus by using 10 ml sodium citrate VACUTAINER.RTM. test-tubes by Kendall, taking care to shake gently just after the drawing of the sample. The so taken blood was immediately subjected to centrifugation (15 min. at 2500 rpm) to separate the platelet rich plasma and adult mesenchymal autologous stem cells. The platelet rich plasma and adult mesenchymal autologous stem cells (12 ml) with all sterility precautions were carefully transferred into four 3 ml sterile syringes. Four, 27 gauge 1 ¼ sterile needles were attached to the sterile syringes containing the platelet rich plasma and adult mesenchymal autologous stem cells. After anesthetizing the area and after all sterility precautions, (12 ml) of platelet rich plasma and adult mesenchymal autologous stem cells were injected into the medial and lateral mensiscus of the patient's left knee. The procedure was performed approximately every three (3) weeks for 7 months and then every (4) weeks for an additional 5 months. MRI 12 months after proved the healing and regeneration of the medial and lateral meniscus, with a better post-operative course than with traditional methods.

Example 3

To produce 6 ml of platelet rich plasma and adult mesenchymal autologous stem cells, obtained as in Example 2, were transferred, with all the measures in order to preserve sterility, into two 3ml syringes according to the present invention, prepared as described in Example 1, from venous blood drawn from a normotype 30 year-old patient with chronic sprain of right ankle involving the anterior and posterior tibiofibular ligaments; the talonavicular ligament and the posterior talofibular ligament. The platelet rich plasma and adult mesenchymal autologous stem cells (6 ml) with all sterility precautions were carefully transferred into four 3 ml sterile syringes. Four, 27 gauge 1 ¼ sterile needles were attached to the sterile syringes containing the platelet rich plasma and adult mesenchymal autologous stem cells. After anesthetizing the area and after all sterility precautions, (6 ml) of platelet rich plasma and adult mesenchymal autologous stem cells were injected into the right ankle as follows: 1.5ml injected into the attachment site of the distal posterior talofibular ligament; 1.5ml injected into the attachment site of the distal medial malleolus; 1.5ml injected into the distal anterior talofibular ligament; 1.5ml injected into the posterior talar process; 1.5ml injected into the sinus tarsi; and 1.5ml injected into the long axis of the metatarsal. The procedure was performed approximately every three (3) weeks for 7 months. MRI 7 months after proved the healing and regeneration of the distal posterior talofibular ligament, distal anterior talofibular ligament and the talocancaneal ligament, with a better post-operative course than with traditional methods.

Example 4

To produce 9 ml of platelet rich plasma and adult mesenchymal autologous stem cells, obtained as in Example 2, were transferred, with all the measures in order to preserve sterility, into three 3ml syringes according to the present invention, prepared as described in Example 1, from venous blood drawn from a normotype 67 year-old patient presenting a right acetabular labrum tear. The platelet rich plasma and adult mesenchymal autologous stem cells (9 ml) with all sterility precautions were carefully transferred into three 3 ml sterile syringes. Three, 25 gauge 2" sterile needles were attached to the sterile syringes containing the platelet rich plasma and adult mesenchymal autologous stem cells. After anesthetizing the area and after all sterility precautions, (9 ml) of platelet rich plasma and adult mesenchymal autologous stem cells were injected into the right hip as follows: 3ml injected into the attachment site of the iliofemoral ligament into the synovial membrane above the greater trochanter; 3ml injected into the attachment site of the iliofemoral ligament into the synovial membrane anterior to the greater trochanter; 3ml injected into the attachment site of the iliofemoral ligament into the synovial membrane posterior to the greater trochanter. The procedure was performed approximately every three (3) weeks for 7 months and then every (4) weeks for an additional 5 months. MRI 12 months after proved marked healing and regeneration of the acetabular labrum and articular cartilage as well as the transverse ligament of the acetabulum, with a better post-operative course than with traditional methods.

Example 5

To produce 9 ml of platelet rich plasma and adult mesenchymal autologous stem cells, obtained as in Example 2, were transferred, with all the measures in order to preserve sterility, into three 3ml syringes according to the present invention, prepared as described in Example 1, from venous blood drawn from a normotype 58 year-old patient presenting a SLAP TEAR (superior labrial tear anterior to posterior) of the left shoulder. The platelet rich plasma and adult mesenchymal autologous stem cells (9 ml) with all sterility precautions were carefully transferred into three, 3 ml sterile syringes. Three, 25 gauge 2" sterile needles were attached to the sterile syringes containing the platelet rich plasma and adult mesenchymal autologous stem cells. After anesthetizing the area and after all sterility precautions, (9 ml) of platelet rich plasma and adult mesenchymal autologous stem cells were injected into the left shoulder as follows: 3ml injected into the site of the anterior glenoidal labrum; 3ml injected into the site of posterior glenoidal labrum; 3ml injected into the site of the superior aspect of the labrum. The procedure was performed approximately every three (3) weeks for 7 months and then every (4) weeks for an additional 5 months. MRI 12 months after proved marked healing and regeneration of the suerior labrum and labrum anteriorly and posteriorly, with a better post-operative course than with traditional methods.

Example 6

To produce 6 ml of platelet rich plasma and adult mesenchymal autologous stem cells, obtained as in Example 2, were transferred, with all the measures in order to preserve sterility, into two 3ml syringes according to the present invention, prepared as described in Example 1, from venous blood drawn from a normotype 34 year-old patient presenting a partial tear of the right lateral collateral ligament. The platelet rich plasma and adult mesenchymal autologous stem cells (6 ml) with all sterility precautions were carefully transferred into two, 3 ml sterile syringes. Two, 27 gauge 1¼" sterile needles were attached to the sterile syringes containing the platelet rich plasma and adult mesenchymal autologous stem cells. After anesthetizing the area and after all sterility precautions, (6 ml) of platelet rich plasma and adult mesenchymal autologous stem cells were injected into the right lateral collateral and right medial collateral ligaments as follows: 1.5ml injected into the site of the ulnar collateral ligament lateral component; 1.5 ml injected into the site of the ulnar collateral ligament posterior component; 1.5l injected into the site of the radial collateral ligament; 1.5ml injected into the site of the annular ligament. The procedure was performed approximately every three (3) weeks for 7 months and then every (4) weeks for an additional 5 months. MRI 12 months after proved marked healing and regeneration of the right lateral collateral ligament, with a better post-operative course than with traditional methods.

Example 7

To produce 6 ml of platelet rich plasma and adult mesenchymal autologous stem cells, obtained as in Example 2, were transferred, with all the measures in order to preserve sterility, into two 3ml syringes according to the present invention, prepared as described in Example 1, from venous blood drawn from a normotype 45 year-old patient presenting degeneration of the cervical vertebra disc and associated cervical facets with particular interest to C4-C5-C6 bilaterally. The platelet rich plasma and adult mesenchymal autologous stem cells (6 ml) with all sterility precautions were carefully transferred into two, 3 ml sterile syringes. Two, 27 gauge 1" sterile needles were attached to the sterile syringes containing the platelet rich plasma and adult mesenchymal autologous stem cells. After anesthetizing the area and after all sterility precautions, (6 ml) of platelet rich plasma and adult mesenchymal autologous stem cells were injected into the cervical facets of C4-C5-C6 as follows: 1ml injected into the site of C4 facet on the right, followed by 1 ml injected into the site of C4 facet on the left, followed by 1ml injected into the site of C5 facet on the right, followed by 1ml injected into the site of C5 facet on the left, followed by 1ml injected into the site of C6 facet on the right, followed by 1ml injected into the C6 facet on the left. The procedure was performed approximately every three (3) weeks for 7 months and then every (4) weeks for an additional 5 months. MRI 12 months after proved marked healing and regeneration of the C4-C5-C6 facets bilaterally, with a better post-operative course than with traditional methods.

Further advantages for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells according to the present invention will be evident to those skilled in the art from the following detailed description of some embodiments thereof and may be utilized on any and all facets of the cervical region.

Example 8

To produce 6 ml of platelet rich plasma and adult mesenchymal autologous stem cells, obtained as in Example 2, were transferred, with all the measures in order to preserve sterility, into two 3ml syringes according to the present invention, prepared as described in Example 1, from venous blood drawn from a normotype 47 year-old patient presenting degeneration of the thoracic vertebra disc and associated thoracic facets with particular interest to T10-T11-12 bilaterally. The platelet rich plasma and adult mesenchymal autologous stem cells (6 ml) with all sterility precautions were carefully transferred into two, 3 ml sterile syringes. Two, 27 gauge 1¼" sterile needles were attached to the sterile syringes containing the platelet rich plasma and adult mesenchymal autologous stem cells. After anesthetizing the area and after all sterility precautions, (6 ml) of platelet rich plasma and adult mesenchymal autologous stem cells were injected into the thoracic facets of T10-T11-T12 as follows: 1ml injected into the site of T10 facet on the right, followed by 1 ml injected into the site of T10 facet on the left, followed by 1ml injected into the site of T11 facet on the right, followed by 1ml injected into the site of T11 facet on the left, followed by 1ml injected into the site of T12 facet on the right, followed by 1ml injected into the T12 facet on the left. The procedure was performed approximately every three (3) weeks for 7 months and then every (4) weeks for an additional 5 months. MRI 12 months after proved marked healing and regeneration of the T10-T11-T12 facets bilaterally, with a better post-operative course than with traditional methods.

Further advantages for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells according to the present invention will be evident to those skilled in the art from the following detailed description of some embodiments thereof and may be utilized on any and all facets of the thoracic region.

Example 9

To produce 12 ml of platelet rich plasma and adult mesenchymal autologous stem cells, obtained as in Example 2, were transferred, with all the measures in order to preserve sterility, into four 3ml syringes according to the present invention, prepared as described in Example 1, from venous blood drawn from a normotype 67 year-old patient presenting degeneration of the lumbo-sacral vertebra disc and associated lumbar facets with particular interest to L4-L5-S1 bilaterally. The platelet rich plasma and adult mesenchymal autologous stem cells (12 ml) with all sterility precautions were carefully transferred into four, 3 ml sterile syringes. Four, 27 gauge 2" sterile

needles were attached to the sterile syringes containing the plasma and adult mesenchymal autologous stem cells. After anesthetizing the area and after all sterility precautions, (12 ml) of platelet rich plasma and adult mesenchymal autologous stem cells were injected into the lumbo-sacral facets of L4-L5-S1 as follows: 2ml injected into the site of L4 facet on the right, followed by 2 ml injected into the site of L4 facet on the left, followed by 2ml injected into the site of L5 facet on the right, followed by 2ml injected into the site of L5 facet on the left, followed by 2ml injected into the site of S1 facet on the right, followed by 2ml injected into the S1 facet on the left. The procedure was performed approximately every three (3) weeks for 7 months and then every (4) weeks for an additional 5 months. MRI 12 months after proved marked healing and regeneration of the L4-L5-S1 facets bilaterally, with a better post-operative course than with traditional methods.

Further advantages for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells according to the present invention will be evident to those skilled in the art from the following detailed description of some embodiments thereof and may be utilized on any and all facets of the lumbar region.

Example 10

To produce 52 ml of platelet rich plasma and adult mesenchymal autologous stem cells, obtained as in Example 2, were transferred, with all the measures in order to preserve sterility, into (14) fourteen 3ml syringes according to the present invention, prepared as described in Example 1, from venous blood drawn from a normotype 59 year-old patient presenting COPD and sarcodosis of related to the lungs and pulmonary system. The platelet rich plasma and adult mesenchymal autologous stem cells (52 ml) with all sterility precautions were carefully transferred into fourteen, 3 ml sterile syringes. Fourteen, 21 gauge 1" sterile needles were attached to the sterile syringes containing the platelet rich plasma and adult mesenchymanl autologous stem cells. Using a nebulizer, 3ml of albuterol, its equivalent, or 3ml of .9% NaCL is placed into the medication. (1) one syringe containing 3ml of platelet rich plasma and adult mesenchymal autologous stem cells is then injected through the 21 gauge 1" needle into the medication cup. The inhalation top is then placed on the medication cup and turned clockwise until securely closed. The container is then gently shaken, to complete dissolution. The desired inhalation accessory (mouthpiece or nosepiece) is then attached to the nebulizer, the power is turned on, the nebulizer begins and the aerosol containing the adult mesenchymal autologous stem cells is generated. Inhalation treatment of platelet rich plasma and adult mesenchymal autologous stem cells was performed for approximately 10-20 minutes each session. The procedure was performed daily for (14) fourteen days.

At the end of (2) two weeks, to produce 42 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated. The procedure was performed every other day for (28) days.

At the end of (4) four weeks, to produce 84 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated. The procedure was performed every third day for (56) days; using a nebulizer, 3ml of albuterol, its equivalent, or 3ml of .9% NaCL was placed into the medication. (1) one syringe containing 3ml of platelet rich plasma and adult mesenchymal autologous stem cells was then injected through the 21 gauge 1" needle into the medication cup. The inhalation top was then placed on the medication cup and turned clockwise until securely closed. The container was then gently shaken, to complete dissolution. The desired inhalation accessory (mouthpiece or nosepiece) was then attached to the nebulizer, the power was turned on, the nebulizer began and the aerosol containing the adult mesenchymal autologous stem cells was generated. Inhalation treatment of adult mesenchymal autologous stem cells was performed for approximately 10-20 minutes each session. The procedure was performed every third day for (56) fifty-six days.

At the end of (8) eight weeks, to produce 96 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (21) twenty-one separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every fourth day for (84) days using a nebulizer, 3ml of albuterol, its equivalent, or 3ml of .9% NaCL was placed into the medication. (1) one syringe containing 3ml of platelet rich plasma and adult mesenchymal autologous stem cells was then injected through the 21 gauge 1" needle into the medication cup. The inhalation top was then placed on the medication cup and turned clockwise until securely closed. The container was then gently shaken, to complete dissolution. The desired inhalation accessory (mouthpiece or nosepiece) was then attached to the nebulizer, the power was turned on, the nebulizer began and the aerosol containing the platelet rich plasma and adult mesenchymal autologous stem cells was generated. Inhalation treatment of platelet rich plasma and adult mesenchymal autologous stem cells was performed for approximately 10-20 minutes each session. The procedure was performed every fourth day for (84) eighty-four days.

At the end of (12) twelve weeks, to produce 51 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (17)seventeen separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every fifth day for (84) days using a nebulizer, 3ml of albuterol, its equivalent, or 3ml of .9% NaCL was placed into the medication. (1) one syringe containing 3ml of platelet rich plasma and adult mesenchymal autologous stem cells was then injected through the 21 gauge 1" needle into the medication cup. The inhalation top was then placed on the medication cup and turned clockwise until securely closed. The container was then gently shaken, to complete dissolution. The desired inhalation accessory (mouthpiece or nosepiece) was then attached to the nebulizer, the power was turned on, the nebulizer began and the aerosol containing the platelet rich plasma and adult mesenchymal autologous stem cells was generated. Inhalation treatment of platelet rich

plasma and adult mesenchymal autologous stem cells was performed for approximately 10-20 minutes each session. The procedure was performed every fifth day for (84) eighty-four days.

At the end of (12) twelve weeks, to produce 42 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (14) fourteen separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every sixth day for (84) days using a nebulizer, 3ml of albuterol, its equivalent, or 3ml of .9% NaCL was placed into the medication. (1) one syringe containing 3ml of platelet rich plasma and adult mesenchymal autologous stem cells was then injected through the 21 gauge 1" needle into the medication cup. The inhalation top was then placed on the medication cup and turned clockwise until securely closed. The container was then gently shaken, to complete dissolution. The desired inhalation accessory (mouthpiece or nosepiece) was then attached to the nebulizer, the power was turned on, the nebulizer began and the aerosol containing the platelet rich plasma and adult mesenchymal autologous stem cells was generated. Inhalation treatment of platelet rich plasma and adult mesenchymal autologous stem cells was performed for approximately 10-20 minutes each session. The procedure was performed every sixth day for (84) eighty-four days. 12 months after proved marked healing and regeneration of the lungs and pulmonary system, with a better post-operative course than with traditional methods.

Further advantages for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells according to the present invention will be evident to those skilled in the art from the following detailed description of some embodiments thereof and may be utilized on any and all systems of a living organism.

Example 11

To produce 42 ml of platelet rich plasma and adult mesenchymal autologous stem cells, obtained as in Example 2, were transferred, with all the measures in order to preserve sterility, into (14) fourteen 3ml syringes according to the present invention, prepared as described in Example 1, from venous blood drawn from a normotype 70 year-old patient presenting aortic stenosis and mitral valve regurgitation related to the heart and cardiovascular system. The platelet rich plasma and adult mesenchymal autologous stem cells (42 ml) with all sterility precautions were carefully transferred into fourteen, 3 ml sterile syringes. Fourteen, 21 gauge 1" sterile needles were attached to the sterile syringes containing the platelet rich plasma and adult mesenchymal autologous stem cells. Using a nebulizer, 3ml of albuterol, its equivalent, or 3ml of .9% NaCL is placed into the medication. (1) one syringe containing 3ml of platelet rich plasma and adult mesenchymal autologous stem cells is then injected through the 21 gauge 1" needle into the medication cup. The inhalation top is then placed on the medication cup and turned clockwise until securely closed. The container is then gently shaken, to complete dissolution. The desired

inhalation accessory (mouthpiece or nosepiece) is then attached to the nebulizer, the power is turned on, the nebulizer begins and the aerosol containing the adult mesenchymal autologous stem cells is generated. Inhalation treatment of platelet rich plasma and adult mesenchymal autologous stem cells was performed for approximately 10-20 minutes each session. The procedure was performed daily for (14) fourteen days.

At the end of (2) two weeks, to produce 42 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated. The procedure was performed every other day for (28) days.

At the end of (4) four weeks, to produce 84 ml of platelet rich plasma and adult mesenchymal autologous, the above procedure was repeated. The procedure was performed every third day for (56) days; using a nebulizer, 3ml of albuterol, its equivalent, or 3ml of .9% NaCL was placed into the medication. (1) one syringe containing 3ml of platelet rich plasma and adult mesenchymal autologous stem cells was then injected through the 21 gauge 1" needle into the medication cup. The inhalation top was then placed on the medication cup and turned clockwise until securely closed. The container was then gently shaken, to complete dissolution. The desired inhalation accessory (mouthpiece or nosepiece) was then attached to the nebulizer, the power was turned on, the nebulizer began and the aerosol containing the platelet rich plasma and adult mesenchymal autologous stem cells was generated. Inhalation treatment of platelet rich plasma and adult mesenchymal autologous stem cells was performed for approximately 10-20 minutes each session. The procedure was performed every third day for (56) fifty-six days.

At the end of (8) eight weeks, to produce 96 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (21) twenty-one separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every fourth day for (84) days using a nebulizer, 3ml of albuterol, its equivalent, or 3ml of .9% NaCL was placed into the medication. (1) one syringe containing 3ml of platelet rich plasma and adult mesenchymal autologous stem cells was then injected through the 21 gauge 1" needle into the medication cup. The inhalation top was then placed on the medication cup and turned clockwise until securely closed. The container was then gently shaken, to complete dissolution. The desired inhalation accessory (mouthpiece or nosepiece) was then attached to the nebulizer, the power was turned on, the nebulizer began and the aerosol containing the adult mesenchymal autologous stem cells was generated. Inhalation treatment of platelet rich plasma and adult mesenchymal autologous stem cells was performed for approximately 10-20 minutes each session. The procedure was performed every fourth day for (84) eighty-four days.

At the end of (12) twelve weeks, to produce 51 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (17)seventeen separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every fifth day

for (84) days using a nebulizer, 3ml of albuterol, its equivalent, or 3ml of .9% NaCL was placed into the medication. (1) one syringe containing 3ml of platelet rich plasma and adult mesenchymal autologous stem cells was then injected through the 21 gauge 1" needle into the medication cup. The inhalation top was then placed on the medication cup and turned clockwise until securely closed. The container was then gently shaken, to complete dissolution. The desired inhalation accessory (mouthpiece or nosepiece) was then attached to the nebulizer, the power was turned on, the nebulizer began and the aerosol containing the adult mesenchymal autologous stem cells was generated. Inhalation treatment of adult mesenchymal autologous stem cells was performed for approximately 10-20 minutes each session. The procedure was performed every fifth day for (84) eighty-four days.

At the end of (12) twelve weeks, to produce 42 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (14) fourteen separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every sixth day for (84) days using a nebulizer, 3ml of albuterol, its equivalent, or 3ml of .9% NaCL was placed into the medication. (1) one syringe containing 3ml of platelet rich plasma and adult mesenchymal autologous stem cells was then injected through the 21 gauge 1" needle into the medication cup. The inhalation top was then placed on the medication cup and turned clockwise until securely closed. The container was then gently shaken, to complete dissolution. The desired inhalation accessory (mouthpiece or nosepiece) was then attached to the nebulizer, the power was turned on, the nebulizer began and the aerosol containing the adult mesenchymal autologous stem cells was generated. Inhalation treatment of platelet rich plasma and adult mesenchymal autologous stem cells was performed for approximately 10-20 minutes each session. The procedure was performed every sixth day for (84) eighty-four days. 12 months after proved marked healing and regeneration of the mitral and aortic valve with no gurgling or stenosis on examination and with a better post-operative course than with traditional methods.

Further advantages for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells according to the present invention will be evident to those skilled in the art from the following detailed description of some embodiments thereof and may be utilized on any and all systems of a living organism.

Example 12

To produce 48 ml of platelet rich plasma and adult mesenchymal autologous stem cells, obtained as in Example 2, were transferred, with all the measures in order to preserve sterility, into (16) sixteen 3ml syringes according to the present invention, prepared as described in Example 1, from venous blood drawn from a normotype 52 year-old patient presenting T.I.A. as a result of Lyme Disease and autoimmune scleroderma. The platelet rich plasma and adult mesenchymal

autologous stem cells (48 ml) with all sterility precautions were carefully transferred into sixteen, 3 ml sterile syringes. Sixteen, 21 gauge 1" sterile needles were attached to the sterile syringes containing the platelet rich plasma and adult mesenchymal autologous stem cells. Using a nebulizer, 3ml of albuterol, its equivalent, or 3ml of .9% NaCL is placed into the medication. (1) one syringe containing 3ml of platelet rich plasma and adult mesenchymal autologous stem cells is then injected through the 21 gauge 1" needle into the medication cup. The inhalation top is then placed on the medication cup and turned clockwise until securely closed. The container is then gently shaken, to complete dissolution. The desired inhalation accessory (mouthpiece or nosepiece) is then attached to the nebulizer, the power is turned on, the nebulizer begins and the aerosol containing the platelet rich plasma and adult mesenchymal autologous stem cells is generated. Inhalation treatment of platelet rich plasma and adult mesenchymal autologous stem cells was performed for approximately 10-20 minutes each session. The procedure was performed daily for (14) fourteen days with the exception of days (7) seven and (14) fourteen.

Day (7) seven, and day (14) fourteen, (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes.

At the end of (2) two weeks, to produce 51 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated. The procedure was performed every other day for (28) days with the exception of days (21) twenty-one, (28) twenty-eight and (42) forty-two , (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session.

At the end of (4) four weeks, to produce 96 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (32) thirty-two separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every third day for (84) days with the exception of days (63) sixty-three, (84) eighty-four, (105) one hundred five and (126) one hundred twenty-six; (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session.

At the end of (12) twelve weeks, to produce 72 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (24) twenty-four separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every fourth day for (84) days with the exception of days (154) one hundred fifty-four, (182) one hundred

eighty-two and (210) two hundred ten, (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session. .

At the end of (12) twelve weeks, to produce 56 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (19) nineteen separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every fifth day for (84) days with the exception of days (252) two hundred fifty-two and (294) two hundred ninety-four, (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session.

At the end of (12) twelve weeks, to produce 48 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (16) sixteen separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every sixth day for (84) days with the exception of days (336) three hundred thirty-six and (379) three hundred seventy-nine, (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session.

Further advantages for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells according to the present invention will be evident to those skilled in the art from the following detailed description of some embodiments thereof and may be utilized on any and all systems of a living organism.

Example 13

To produce 48 ml of platelet rich plasma and adult mesenchymal autologous stem cells, obtained as in Example 2, were transferred, with all the measures in order to preserve sterility, into (16) sixteen 3ml syringes according to the present invention, prepared as described in Example 1, from venous blood drawn from a normotype 38 year-old patient presenting multiple sclerosis with a history of (10) ten years. The platelet rich plasma and adult mesenchymal autologous stem cells (48 ml) with all sterility precautions were carefully transferred into sixteen, 3 ml sterile syringes. Sixteen, 21 gauge 1" sterile needles were attached to the sterile syringes containing the platelet rich plasma and adult mesenchymal autologous stem cells. Using a nebulizer, 3ml of albuterol, its equivalent, or 3ml of .9% NaCL is placed into the medication. (1) one syringe

containing 3ml of plasma and adult mesenchymal autologous stem cells is then injected through the 21 gauge 1" needle into the medication cup. The inhalation top is then placed on the medication cup and turned clockwise until securely closed. The container is then gently shaken, to complete dissolution. The desired inhalation accessory (mouthpiece or nosepiece) is then attached to the nebulizer, the power is turned on, the nebulizer begins and the aerosol containing the platelet rich plasma and adult mesenchymal autologous stem cells is generated. Inhalation treatment of platelet rich plasma and adult mesenchymal autologous stem cells was performed for approximately 10-20 minutes each session. The procedure was performed daily for (14) fourteen days with the exception of days (7) seven and (14) fourteen.

Day (7) seven, and day (14) fourteen, (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes.

At the end of (2) two weeks, to produce 51 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated. The procedure was performed every other day for (28) days with the exception of days (21) twenty-one, (28) twenty-eight and (42) forty-two , (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session.

At the end of (4) four weeks, to produce 96 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (32) thirty-two separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every third day for (84) days with the exception of days (63) sixty-three, (84) eighty-four, (105) one hundred five and (126) one hundred twenty-six; (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session.

At the end of (12) twelve weeks, to produce 72 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (24) twenty-four separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every fourth day for (84) days with the exception of days (154) one hundred fifty-four, (182) one hundred eighty-two and (210) two hundred ten, (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session. .

At the end of (12) twelve weeks, to produce 56 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the adult mesenchymal autologous stem cells into (19) nineteen separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every fifth day for (84) days with the exception of days (252) two hundred fifty-two and (294) two hundred ninety-four, (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session.

At the end of (12) twelve weeks, to produce 48 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (16) sixteen separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every sixth day for (84) days with the exception of days (336) three hundred thirty-six and (379) three hundred seventy-nine, (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session.

Further advantages for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells according to the present invention will be evident to those skilled in the art from the following detailed description of some embodiments thereof and may be utilized on any and all systems of a living organism.

Example 14

To produce 48 ml of platelet rich plasma and adult mesenchymal autologous stem cells, obtained as in Example 2, were transferred, with all the measures in order to preserve sterility, into (16) sixteen 3ml syringes according to the present invention, prepared as described in Example 1, from venous blood drawn from a normotype 45 year-old patient presenting Parkinson's Disease with a history of (10) ten years. The platelet rich plasma and adult mesenchymal autologous stem cells (48 ml) with all sterility precautions were carefully transferred into sixteen, 3 ml sterile syringes. Sixteen, 21 gauge 1" sterile needles were attached to the sterile syringes containing the platelet rich plasma and adult mesenchymal autologous stem cells. Using a nebulizer, 3ml of albuterol, its equivalent, or 3ml of .9% NaCL is placed into the medication. (1) one syringe containing 3ml of platelet rich plasma and adult mesenchymal autologous stem cells is then injected through the 21 gauge 1" needle into the medication cup. The inhalation top is then placed on the medication cup and turned clockwise until securely closed. The container is then gently shaken, to complete dissolution. The desired inhalation accessory (mouthpiece or

nosepiece) is then attached to the nebulizer, the power is turned on, the nebulizer begins and the aerosol containing the adult mesenchymal autologous stem cells is generated. Inhalation treatment of platelet rich plasma and adult mesenchymal autologous stem cells was performed for approximately 10-20 minutes each session. The procedure was performed daily for (14) fourteen days with the exception of days (7) seven and (14) fourteen.

Day (7) seven, and day (14) fourteen, (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes.

At the end of (2) two weeks, to produce 51 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated. The procedure was performed every other day for (28) days with the exception of days (21) twenty-one, (28) twenty-eight and (42) forty-two , (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session.

At the end of (4) four weeks, to produce 96 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (32) thirty-two separate 3ml sterile splayringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every third day for (84) days with the exception of days (63) sixty-three, (84) eighty-four, (105) one hundred five and (126) one hundred twenty-six; (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session.

At the end of (12) twelve weeks, to produce 72 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (24) twenty-four separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every fourth day for (84) days with the exception of days (154) one hundred fifty-four, (182) one hundred eighty-two and (210) two hundred ten, (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session. .

At the end of (12) twelve weeks, to produce 56 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (19) nineteen separate 3ml sterile syringes,

each with (1) 1" sterile needle attached to syringe. The procedure was performed every fifth day for (84) days with the exception of days (252) two hundred fifty-two and (294) two hundred ninety-four, (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session.

At the end of (12) twelve weeks, to produce 48 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (16) sixteen separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every sixth day for (84) days with the exception of days (336) three hundred thirty-six and (379) three hundred seventy-nine, (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session.

Further advantages for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells according to the present invention will be evident to those skilled in the art from the following detailed description of some embodiments thereof and may be utilized on any and all systems of a living organism.

Example 15

To produce 48 ml of platelet rich plasma and adult mesenchymal autologous stem cells, obtained as in Example 2, were transferred, with all the measures in order to preserve sterility, into (16) sixteen 3ml syringes according to the present invention, prepared as described in Example 1, from venous blood drawn from a normotype 65 year-old patient presenting COPD and macular degeneration with a history of (15) fifteeen years. The platelet rich plasma and adult mesenchymal autologous stem cells (48 ml) with all sterility precautions were carefully transferred into sixteen, 3 ml sterile syringes. Sixteen, 21 gauge 1" sterile needles were attached to the sterile syringes containing the platelet rich plasma and adult mesenchymal autologous stem cells. Using a nebulizer, 3ml of albuterol, its equivalent, or 3ml of .9% NaCL is placed into the medication. (1) one syringe containing 3ml of platelet rich plasma and adult mesenchymal autologous stem cells is then injected through the 21 gauge 1" needle into the medication cup. The inhalation top is then placed on the medication cup and turned clockwise until securely closed. The container is then gently shaken, to complete dissolution. The desired inhalation accessory (mouthpiece or nosepiece) is then attached to the nebulizer, the power is turned on, the nebulizer begins and the aerosol containing the platelet rich plasma and adult mesenchymal autologous stem cells is generated. Inhalation treatment of platelet rich plasma and adult

mesenchymal autologous stem cells was performed for approximately 10-20 minutes each session. The procedure was performed daily for (14) fourteen days with the exception of days (7) seven and (14) fourteen.

Day (7) seven, and day (14) fourteen, (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes.

At the end of (2) two weeks, to produce 51 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated. The procedure was performed every other day for (28) days with the exception of days (21) twenty-one, (28) twenty-eight and (42) forty-two , (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session.

At the end of (4) four weeks, to produce 96 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (32) thirty-two separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every third day for (84) days with the exception of days (63) sixty-three, (84) eighty-four, (105) one hundred five and (126) one hundred twenty-six; (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session.

At the end of (12) twelve weeks, to produce 72 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (24) twenty-four separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every fourth day for (84) days with the exception of days (154) one hundred fifty-four, (182) one hundred eighty-two and (210) two hundred ten, (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session. .

At the end of (12) twelve weeks, to produce 56 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (19) nineteen separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every fifth day for (84) days with the exception of days (252) two hundred fifty-two and (294) two hundred

ninety-four, (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session.

At the end of (12) twelve weeks, to produce 48 ml of platelet rich plasma and adult mesenchymal autologous stem cells, the above procedure was repeated, placing 3ml of the platelet rich plasma and adult mesenchymal autologous stem cells into (16) sixteen separate 3ml sterile syringes, each with (1) 1" sterile needle attached to syringe. The procedure was performed every sixth day for (84) days with the exception of days (336) three hundred thirty-six and (379) three hundred seventy-nine, (6) six ml of platelet rich plasma and adult mesenchymal autologous stem cells were injected into an IV bag containing 250ml of .9% NaCL at room temperature and gently shaken to complete dissolution. The solution was then injected intravenously into the patient for approximately (45) forty-five minutes at each session.

Further advantages for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells according to the present invention will be evident to those skilled in the art from the following detailed description of some embodiments thereof and may be utilized on any and all systems of a living organism.

Example 16

To produce 36 ml of platelet rich plasma and adult mesenchymal autologous stem cells, obtained as in Example 2, were transferred, with all the measures in order to preserve sterility, into twelve 3ml syringes according to the present invention, prepared as described in Example 1, from venous blood drawn from a normotype 24 year-old patient. The platelet rich plasma and adult mesenchymal autologous stem cells (36 ml) with all sterility precautions were carefully transferred into twelve, 3 ml sterile syringes. Twelve, 21 gauge 1" sterile needles were attached to the sterile syringes containing the platelet rich plasma and adult mesenchymal autologous stem cells. 3ml of platelet rich plasma and adult mesencymal autologous stem cells with all sterility precautions were carefully placed into a petrie dish containing a serum free culture medium specifically formulated for the culture of pure populated adult mesenchymal autologous stem cells. By blocking protein membrane CD47 variations of the adult mesencymal stem cell may be grown into specific cells.

Further advantages for deriving, preparing and administering adult mesenchymal autologous stem cells according to the present invention will be evident to those skilled in the art from the following detailed description of some embodiments thereof and may be utilized on any and all systems of a living organism.

Generally speaking, the invention provides integrated systems and methodologies for deriving, preparing and administering adult mesenchymal autologous stem cells suitable for regenerating tissue in a living organism without resulting in viral infections and/or rejection cases when used in oral, injectibles, intravenously, intra-arterial, minor and major surgery.

In one embodiment (shown in FIG. 1), the system comprises a primary container 10 capable of removing blood 11from a living organism into a secondary container 12. Preferably, the secondary container 12, are tubes, and more particularly, test tubes, although any container that is capable of holding a fluid or liquid and being centrifuged is suitable for use with the invention. Preferably, the container 12, is made from glass or plastics.

The primary container 10 must be capable of drawing blood therein using standard venipuncture techniques into secondary container 12. Preferably the secondary container 12 is sealed with a seal 13 while the blood is being drawn to prevent contamination, although the container 12 may be sealed shortly thereafter. A variety of seals 13 can be used to seal the secondary container 12, e.g., a rubber stopper, cap, foam, elastomer or other composite. The seal 13 should be capable of being pierced or punctured, and therefore rubber and silicone are preferred materials from which the seal is fabricated, although any material that provides a seal and is capable of being pierced can be used.

In another embodiment (shown in FIG. 3), the secondary container 12 may contain an anticoagulant solution 14. The anticoagulant 14 may comprise sodium citrate, ethylenelendiaminetetraacetic acid disodium salt, ethylenelendiaminetetraacetic acid dipotassium salt and tripotassium and combinations thereof. Preferably, the secondary container 12 contains a sodium citrate solution. The anticoagulant 14 tends to thin blood collected in the secondary container 12 in order to place it in condition for centrifugation.

In another embodiment, the secondary container may also contain one or more of an antibiotic, an analgesic, a cancer therapeutic, a platelet-growth factor and a bone morphogenic protein. Other therapeutic agents which can be topically administered may also be included. Examples of antibiotics include, but are not limited to, ampicillin, erythromycin and tobramycin. Analgesics include, but are not limited to, aspirin and codeine. Cancer therapeutics include, but are not limited to, 5-fluor-uracile.

Turning now to the operation of the first embodiment, once blood has been drawn into the secondary container 12 using standard venipuncture techniques, the blood is anticoagulated by the anti-coagulant 14 therein. Typically, the secondary container 12 is sealed while the blood is being drawn, however, it may be sealed thereafter. Sealing the secondary container 12 prevents contamination of the contents therein.

Thereafter, the secondary container 12 and its contents 11 (i.e. blood, anti-coagulant 14) are centrifuged. Acceptable centrifugation can take place at a gravitational force in the range of 900 to 3,500 xG for 5 to 20 minutes. In a preferred embodiment, the secondary container is centrifuged at a gravitational force of about 3,000 xG for about fifteen minutes. This initial centrifugation separates the secondary container's contents or fractions into a plurality of layers as shown, e.g., in FIG.9.

The layers include (in order from the bottom of the secondary container 12 to the top of the container after centrifugation) as shown, e.g., in FIG 9: the red blood cell layer 15, the buffy layer containing adult mesenchymal autologous stem cells 16, the platelet-rich plasma layer 17, and finally a residual gas 18 volume at a pressure equal to atmospheric. The proportions of these layers may vary from application to application, and are shown here in these proportions for illustrative purposes only. Subsequent to centrifugation, the sealed secondary holder 12 is placed into the transfer device 19 is used to house the secondary container 12 in the centrifuge.

In other words, the secondary container 12 is placed in the centrifuge such that the sealed opening is in the highest vertical position as shown in FIG. 8. Placing the secondary container in such a manner allows gravity to control the order in which the layers are arranged. Below the seal 13 are the following layers in sequence from top to bottom after centrifuge as shown, e.g., in FIG 9 : residual gas18, platelet rich plasma 17, buffy layer containing adult mesenchymal autologous stem cells 16, and the red blood cells 15.

Next, the secondary container 12 is placed in a vertical position with its sealed opening 13 in the top most position as best shown in FIG. 8. This positions the secondary container 12 for the centrifuging of the secondary container's contents therein. FIG. 8 illustrates the positioning of the secondary container 12 to be centrifuged in a vertical position above the centrifuge 23, which is below the secondary container 12 in the proper position for transfer.

Because of the particular sequential arrangement of the layers in the secondary container 12, the platelet-rich plasma 17 and the adult mesenchymal autologous stem cells 16 are easily transferred. In addition, because the secondary container 12 is also preset to an evacuation level, the container only partially fills after blood collection. This allows the gas in the "head space" to remain significantly above zero during transfer when its volume is expanded, thereby allowing fast and complete transfer into the secondary container 12. This is dictated by the ideal gas law and the Poiseuille-Hagen equation.

Transfer of the contents or fragments of the secondary container (i.e. the platelet-rich plasma and adult mesenchymal autologous stem cells) continues into the collecting device with a housing and a member 20, wherein the member is moveable in a distal direction in order to transfer the medium until only the red blood cells remain in the secondary container 12. The transfer is performed in such a way that it also prevents accidental contamination by blood borne pathogens by prior use on or by another patient.

The transfer of the plasma fraction consisting of the platelet-rich plasma 17 and adult mesenchymal autologous stem cells 16 to the collecting device with a housing and a member 20 is complete, thereby allowing maximum yield and maintenance of the appropriate mediums. The collecting device 20 is then able to dispense its medium into an area that includes but is not limited to; musculoskeletal 24, ligament 25, tendon 26, cartilage 27, meniscus 28, disc 29(FIG.16), and organ of a living organism for regeneration of said area and/or; the container containing the medium of plasma, adult mesenchymal autologous stem cells and sodium chloride or similar medium can now be dispensed into a living organism via intravenous or intra-arterial (FIG. 17); or, the container containing the medium of plasma, adult mesenchymal autologous stem cells and albuterol or sodium chloride or similar medium can now be dispensed into a living organism via inhalation by way of nebulizer (FIG. 19); or, the container containing the medium of plasma is able to dispense its medium into a second medium housed in a container 30 (FIG. 13) for multiplying, regenerating, duplicating and incubating adult mesenchymal autologous stem cells for future use. The transfer occurs without venting, maintaining sterility and non-contamination of the sample.

The invention also provides a ready-to-use step-by-step system and methodology for deriving, preparing and administering adult mesenchymal autologous stem cells suitable for regenerating tissue in a living organism as shown in FIG. 20. The methodology comprises a primary container 10, the secondary container 12 and the transfer device 10.

In one embodiment, the system is packaged in such a way that the accoutrements include all the components required for deriving, preparing and administering adult mesenchymal autologous stem cells suitable for regenerating tissue in a living organism as shown in FIG. 20. Of course, the components can be arranged in a wide variety of manners.

In one aspect, the invention provides a system and methodology for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells suitable for regenerating tissue in a living organism.

Step 1 comprises collecting blood into the primary container 10 into the secondary container 12, followed by centrifugation to obtain platelet rich plasma and adult mesenchymal autologous stem cells. The components for the initiation of the process comprise an alcohol swab 34 to cleanse the venipuncture site, a multiple sample blood collection needle 10 as part of the primary container (21 gauge.times.1"), the secondary container 12 containing the anticoagulant (e.g. citrate), and a bandage 35 to cover the venipuncture site. The venipuncture site is cleansed with the sterile alcohol swab 34. The needle cover is removed from the end of the primary container. The needle at the end of the primary container 10 is then inserted into the patient's vein and the opposite end of the primary container 10 is inserted into the seal 13 of the secondary container 12. Blood then fills the secondary container12, after which the needle is withdrawn from the seal 13 of the secondary container and afterwards the needle is removed from the patient's vein and retracted into its holder. The vein is closed with the bandage 35. The secondary container 12 is

placed in a vertical position with its sealed opening 13 in the top most position as best shown in FIG. 8. This positions the secondary container 12 for the centrifuging of the secondary container's contents therein. FIG. 8 illustrates the positioning of the secondary container 12 to be centrifuged in a vertical position above the centrifuge 23, which is below the secondary container 12 in the proper position for transfer.

Because of the particular sequential arrangement of the layers in the secondary container 12, the platelet rich plasma 17 and the adult mesenchymal autologous stem cells 16 are easily transferred. In addition, because the secondary container 12 is also preset to an evacuation level, the container only partially fills after blood collection. This allows the gas in the "head space" to remain significantly above zero during transfer when its volume is expanded, thereby allowing fast and complete transfer into the secondary container 12. This is dictated by the ideal gas law and the Poiseuille-Hagen equation.

The container 12 is centrifuged at about 3000 xG for about 15 minutes and the platelet rich plasma and adult mesenchymal autologous stem cells are separated from the red blood cells.

The secondary container after being centrifuged contains residual gas 18, platelet rich plasma 17, buffy layer with adult mesecnhymal autologous stem cells16 and red blood cells 15.

The components of the second step in the methodology for deriving, preparing and administering platelet rich plasma and adult mesenchymal autologous stem cells suitable for regenerating tissue in a living organism include a collecting device with a housing and a member 20 is complete, thereby allowing maximum yield and maintenance of the appropriate mediums.

Transfer of the contents or fragments of the secondary container (i.e. the platelet rich plasma and adult mesenchymal autologous stem cells) continues into the collecting device with a housing and a member 20, that has a the member that is moveable in a distal direction in order to transfer the medium until only the red blood cells remain in the secondary container 12. The transfer is performed in such a way that it also prevents accidental contamination by blood borne pathogens by prior use on or by another patient.

The transfer of the plasma fraction consisting of the platelet rich plasma 17 and adult mesenchymal autologous stem cells 16 to the collecting device with a housing and a member 20 is complete, thereby allowing maximum yield and maintenance of the appropriate mediums. The collecting device 20 is then able to dispense its medium into an area that includes but is not limited to; musculoskeletal 24, ligament 25, tendon 26, cartilage 27, meniscus 28, disc 29(FIG.16), and organ of a living organism for regeneration of said area and/or; the container containing the medium of platelet rich plasma, adult mesenchymal autologous stem cells and sodium chloride or similar medium can now be dispensed into a living organism via intravenous or intra-arterial (FIG. 17); or, the container containing the medium of plasma, adult

mesenchymal autologous stem cells and sodium chloride or similar medium can now be dispensed into a living organism via inhalation by way of nebulizer (FIG. 19); or, the container containing the medium of plasma is able to dispense its medium into a second medium housed in a container 30 (FIG. 13) for multiplying, regenerating, duplicating and incubating adult mesenchymal autologous stem cells for future use. The transfer occurs without venting, maintaining sterility and non-contamination of the sample.

In a second embodiment, the chamber 32 may also contain one or more of an antibiotic, an analgesic, a cancer therapeutic, a platelet-growth factor and a bone morphogenic protein. Other therapeutic agents which can be topically administered may also be included. Examples of antibiotics include, but are not limited to, ampicillin, erythromycin and tobramycin. Analgesics include, but are not limited to, aspirin and codeine. Cancer therapeutics include, but are not limited to, 5-fluor-uracile.

In operation, a patient's blood 11 is collected into the secondary collection device 12 by conventional venipuncture technique as described above. The anticoagulant in the primary collection device 12 thins the blood before centrifugation.

In that embodiment, the reservoir 32 may also contain one or more of an antibiotic, an analgesic, a cancer therapeutic, a platelet-growth factor and a bone morphogenic protein. Other therapeutic agents which can be topically administered may also be included. Examples of antibiotics include, but are not limited to, ampicillin, erythromycin and tobramycin. Analgesics include, but are not limited to, aspirin and codeine. Cancer therapeutics include, but are not limited to, 5-fluor-uracile.

I, Jimmy Lee Taylor, do hereby transfer & relinquish to my only heir & beneficiary, Zachary Sebastian Taylor, the following:

1) All proprietary patents (listed on separate page) & intellectual property in my name, or that of my non-profit American Hospitals Intl., pertaining to stem cell IP, including, but not limited to, white pages and clinical studies on disease & treatment research, proprietary formulas and chemical solutions, and proprietary natural stem cell & hormone extraction techniques.

2) Specialized Power of Attorney to act on my behalf, whether or not I am incapacitated, in matters of, but not limited to, banking and purchasing.

3) Any liquid assets in bank accounts, foreign & domestic (listed on separate page).

4) Any and all proceeds from my non-profit American Hospitals Intl, which I would otherwise receive.

This agreement is lawfully binding and supersedes and overrides any previous such agreements.

Dr. Jim Taylor
4/30/18

ebastian Taylor - WITNESS

- WITNESS