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(54) **Title:** CELL PROCESSING METHOD AND DEVICE

(57) **Abstract:** The present invention relates to methods of processing adipose tissue to generate a cell suspension, and devices suitable for use in such methods. The invention also relates to pharmaceutical compositions of adipose tissue derived cell suspensions.



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Cell processing method and device

Field

The present invention relates to methods of processing adipose tissue to generate a
5 cell suspension, and devices suitable for use in such methods.

Background

Adipocytes are cells which lie within adipose tissue and which serve a role of storing fat. It has recently been recognized that adipocytes also play an important cell-signaling role, though the secretion of a range of hormones and cytokines by these cells.

10 Networks of capillaries throughout adipose tissue deliver nutrients and oxygen to the adipocytes and carry away secretions. Large numbers of cells that are not adipocytes, including mesenchymal stem cells, leukocytes, endothelial cells, vascular smooth muscle cells, pericytes, granulocytes, hematopoietic stem cells, monocytes, T cells, mast cells and B cells, are also associated with the capillaries of adipose tissue. Many of these cell types
15 may be involved in angiogenesis and may secrete anti-inflammatory cytokines.

Adipose tissue may be harvested to isolate stem cells, adipocytes and/or other cells for regenerative medicine purposes. In humans adipose tissue is typically harvested by liposuction. In animals such as dogs and horses adipose tissue is normally harvested by surgical excision and the tissue then minced.

20 Blood cells are preferably removed from the liposuction aspirate or minced adipose tissue before the sample is processed to isolate cells as they may interfere with the cell isolation process. The removal of blood cells by extensive rinsing of tissue is laborious and during this process it is difficult to maintain sterility.

Cells are isolated from liposuction aspirates or minced adipose tissue by mixing the
25 washed adipose tissue with one or more proteolytic enzymes, such as collagenase, and allowing enzymic liberation of cells from the tissue.

After enzymic digestion filtration is required to remove undigested large tissue pieces. The digested sample is typically also centrifuged to pellet the cells and to allow the proteolytic enzymes to be removed. The sample may be filtered either before or after
30 the centrifugation procedure. The cell suspension is then ready for use, such as for injection into the recipient subject.

There is a need for devices and techniques which streamline and/or simplify the digestion of adipose tissue to allow the rapid generation of an adipose tissue derived cell suspension.

Summary

5 The methods described herein allow the collection and generation of an adipose tissue derived cell suspension without requiring transfer of the adipose tissue from the collection vessel.

Accordingly provided herein is a method of generating an adipose tissue derived cell suspension from collected adipose tissue, comprising:

- 10 (i) collecting adipose tissue in a collection vessel,
the collection vessel comprising a cell permeable screen which separates an inner chamber and an outer chamber within the collection vessel, at least one port openable from outside the collection vessel into the inner chamber and at least one port openable from outside the collection vessel into the outer chamber, the adipose tissue being
15 collected into either the inner chamber or the outer chamber of the collection vessel;
(ii) optionally washing the adipose tissue within the collection vessel;
(iii) exposing the adipose tissue to a proteolytic enzyme solution to generate a cell suspension; and
(iv) collecting the adipose tissue cell suspension from the chamber which is other
20 than the chamber in which the adipose tissue was collected.

In an embodiment the collection vessel is suitable for use in a centrifuge. centrifugation of a cell suspension. In an embodiment an inner surface of said collection vessel comprises a conical shape suitable for centrifugation of a cell suspension within said vessel.

- 25 In an embodiment the method further comprises, prior to said collecting in step (iv), centrifuging said vessel to fractionate components of said cell suspension to form a cell pellet, a cell layer which comprises adipocytes, an intermediate layer which is depleted of cells relative to the cell pellet and the adipocyte layer, and, where present, a free lipid layer; and removing the intermediate layer and, where present, the free lipid layer through
30 a port openable from outside the collection vessel, thereby retaining within said vessel an adipose tissue-derived cell suspension comprising adipocytes. In an embodiment, said removing is through a port openable from outside the collection vessel into the chamber into which the adipose tissue was collected. In an embodiment said removing is through a

port openable from outside the collection vessel into the chamber which is other than the chamber into which the adipose tissue was collected.

In an embodiment, the method further comprises, after said centrifugation and removing, washing the remaining cell suspension by addition of a suitable liquid and
5 repeating said centrifugation and removing.

In an embodiment the method further comprises, after said collecting in step (iv), transferring said collected adipose tissue cell suspension to a fractionation vessel and centrifuging said vessel to form a cell pellet, a cell layer which comprises adipocytes, an intermediate layer which is depleted of cells relative to the cell pellet and the adipocyte
10 layer, and, where present, a free lipid layer; and removing the intermediate layer and, where present, the free lipid layer; thereby retaining within said fractionation vessel an adipose tissue-derived cell suspension comprising adipocytes. In an embodiment the fractionation vessel is a centrifuge tube or a centrifuge bottle.

In an embodiment the method further comprises, after centrifugation of contents of
15 the vessel, partially or completely removing a cell layer which comprises adipocytes, thereby retaining within the vessel an adipose tissue-derived cell suspension having a reduced content of adipocytes compared to the material prior to said centrifugation. In an embodiment the adipose tissue-derived cell suspension is substantially free of adipocytes.

In an embodiment the method further comprises, after centrifugation of contents of
20 the vessel, collecting a cell pellet by selective removal of the cell pellet from the vessel, and resuspending the collected cell pellet in a suitable liquid to constitute an adipose tissue-derived cell suspension having a reduced content of adipocytes compared to the material prior to said centrifugation.

In an embodiment one or more of a liquid for washing the adipose tissue and the
25 proteolytic enzyme solution is warmed to 37°C prior to contact with said adipose tissue.

In an embodiment one or more of said washing or said exposing steps is conducted in a temperature controlled environment. In an embodiment said temperature controlled environment maintains a temperature within said vessel of about 37°C. In an embodiment the temperature controlled environment is an insulated enclosure and a heat source.

30 In an embodiment exposing the adipose tissue to a proteolytic enzyme solution to generate a cell suspension further comprises constant or intermittent agitation of the adipose tissue and proteolytic enzyme solution.

In one embodiment, the step of collecting adipose tissue comprises liposuction.

In one embodiment the proteolytic enzyme solution comprises collagenase. In one embodiment the collagenase in the exposing step is at a concentration between about 0.01%w/v and about 0.25%w/v.

Also provided herein is a tissue processing system comprising:

- 5 - a collection vessel comprising a cell permeable screen which separates an inner chamber and an outer chamber within the collection vessel, at least one port openable from outside the collection vessel into the inner chamber and at least one port openable from outside the collection vessel into the outer chamber, and
- 10 - a fractionation vessel comprising at least two ports openable from outside the fractionation vessel to inside, whereby in use at least one of said ports is detachably connected to at least one port of said collection vessel.

In one embodiment, said fractionation vessel comprises a solid covering over the vessel, through which said at least two openable ports are located. In one embodiment,
15 said solid covering is not removable from said vessel. In one embodiment, an internal opening of at least one of said openable ports is positioned at a lower portion within the fractionation vessel. In one embodiment said internal opening is located at a lower portion within said fractionation vessel at the end of a U-shaped bend, wherein the attitude of the opening is away from the bottom of the vessel.

20 In one embodiment a port openable from outside said collection vessel or said fractionation vessel is created by separation of said collection and said fractionation vessels.

In one embodiment at least one of said ports openable from outside said collection vessel or said fractionation vessel, respectively, serves also as port openable from outside
25 said fractionation vessel or said collection vessel, respectively.

In one embodiment said collection vessel and said fractionation vessel are detachably connected to form a single vessel.

In one embodiment said collection vessel and said fractionation vessel when connected comprise distinct functional regions within a single connected vessel.

30 Also provided according to the invention is the use of a tissue processing system or device according to the invention for the preparation of an adipose tissue-derived cell suspension.

Also provided according to the invention is an adipose tissue-derived cell suspension when generated according to the method of the invention, for use in the
35 preparation of a pharmaceutical composition.

The use of a system of the invention permits adipose tissue collection, optionally washing, digestion to form a cell suspension and centrifugation of said cell suspension to all occur within a controlled environment, which in a preferred embodiment may be a closed environment, thereby reducing handling and transfer of the adipose tissue and cell suspension with resultant improvement in efficiency and sterility of tissue processing.

Also provided herein is a tissue processing vessel comprising a cell permeable screen which separates an inner chamber and an outer chamber within the vessel, at least one port openable from outside the vessel into the inner chamber and at least one port openable from outside the vessel into the outer chamber, wherein the vessel is suitable for use in a centrifuge.

In an embodiment an inner surface of said vessel comprises a conical shape suitable for centrifugation of a cell suspension within said vessel.

In an embodiment the outer base of the vessel is flat. In an embodiment the inner base of the vessel is conical.

In an embodiment the vessel comprises two ports openable from outside the vessel into the inner chamber. In an embodiment the vessel comprises three ports openable from outside the vessel into the inner chamber. In an embodiment the vessel comprises two ports openable from outside the vessel into the outer chamber.

In an embodiment one or more of said ports comprises an opening within the region of the vessel defined by the conical shape. In an embodiment one or more of said ports comprises an opening within said vessel suitable for the removal of an intermediate layer which is depleted of cells relative to a cell pellet and a floating adipocyte layer following centrifugation of an adipose tissue cell suspension. In an embodiment one or more of said ports comprises an opening within said vessel suitable for the removal of a free lipid layer following centrifugation of an adipose tissue cell suspension. In an embodiment an opening of one or more of said ports within said vessel is comprised in a detachable tube fitted to said port. In an embodiment one or more of the ports is adjustable in height and can be lowered or raised inside the vessel.

Also provided herein is the use of a tissue processing vessel of the invention for the preparation of an adipose tissue-derived cell suspension.

The summary of the invention described above is not limiting and other features and advantages of the invention will be apparent from the following detailed description of the preferred embodiments, as well as from the claims.

Brief description of the Figures

Preferred forms of the present invention will now be described with reference to the accompanying drawings in which:

- 5 **Figure 1A** is a schematic cross section of one embodiment of a device suitable for collecting and digesting adipose tissue according to the methods described herein. The device comprises a sterilizable collection vessel (100) for holding adipose tissue, and for digesting the adipose tissue with a solution comprising proteolytic enzymes, liberated cells, and rinsing solutions. An optional removable lid section (110) allows access to the
- 10 inside of the collection chamber, for example to allow cleaning if the chamber is to be re-used. A cell permeable screen (120) separating an inner chamber (140) and an outer chamber (150) allows solution comprising cells or small pieces of digested tissue to pass between the inner and outer chambers, whilst retaining large tissue pieces within the inner chamber.
- 15 Ports (130) allow the addition and/or removal of tissue, cells and solutions into the inner and/or outer chambers of the collection chamber. Optionally, any one or more of the ports (130) may be adjustable in height allowing them to be lowered or raised inside the vessel as desired. In this embodiment a collection port (132) opens to the outer chamber, with the opening extended by a tube towards the bottom of the collection vessel
- 20 so that liquids lying at the bottom of the vessel may be aspirated. A vacuum line (not shown) may be placed on the collection port if the collection chamber is used to collect adipose tissue during liposuction. The collection port also allows collection of a cell suspension which is generated from enzymic digestion of adipose tissue in the inner chamber.
- 25 In this embodiment two delivery ports (134) and (136) open to the inner chamber, allowing the addition of adipose tissue, rinsing solution, and enzyme solution into the inner chamber. One of the delivery ports may be connected to a liposuction cannula (not shown) and thus may serve as the port of entry of adipose tissue into the collection chamber during liposuction.
- 30 The device illustrated in Figure 1A may be used alone in a method of the invention for the collection of adipose tissue, for optional washing of the adipose tissue, and for digestion of the adipose tissue. The device illustrated in Figure 1A may also be used as part of a system for processing of adipose tissue, as described for example in the following sections.

Figure 1B is a schematic cross section of one embodiment of a conjugate device suitable for use in a system for collecting and digesting adipose tissue and centrifugation to fractionate or further process desired components of the cell suspension derived from adipose tissue. The device shown in Figure 1B may be referred to as the fractionation vessel (1.0) and may be used as part of a system comprising a compatible device suitable for collection and digestion of adipose tissue, such as the device illustrated in Figure 1A. In the illustrated embodiment the fractionation vessel comprises a sterilizable vessel (1.0) which comprises a solid covering (1.3), which is not removable, at one end. The vessel (1.0) is conical shaped at the end distal to the solid covering, for greater compatibility with use in centrifugation. In alternative embodiments the cover (1.3) may be removable, for example to allow access to the inside of the vessel, for example to allow cleaning if the vessel is to be re-used. External ports (1.1 and 1.2) are compatible with the connection of a luer lock. Ports (1.1 and 1.2) allow the addition and/or removal of cells and solutions into the vessel. Port (1.2) may alternatively be connected to a vacuum source. Port (1.2) is connected to opening (1.4) within the fractionation vessel. Opening (1.4) is flush with the inner surface of the solid covering of the vessel. This flush finish permits ease of removal of contents from the vessel through opening (1.4) by connection of, for example, a syringe to port (1.2) and the inversion of the vessel so all contents lie against the inner surface within which opening (1.4) lies. Port (1.1) connects to the inner space of the vessel via tube (1.5) which is standard diameter hosing with a central facing U-bend distal to opening (1.1). Tube (1.5) extends to a lower portion of the vessel to open into the vessel through opening (1.6). Opening (1.6) is positioned such that it lies within the liquid phase after centrifugation of a cell suspension. In operation opening (1.6) thus permits the liquid phase immediately above a cell pellet after centrifugation to be aspirated. A vacuum line (not shown) may be placed on port (1.1) to permit removal of that liquid phase.

In this embodiment port (1.1) also serves as the delivery port for delivery of the cell suspension, such as by vacuum line (not shown) connected to a source of the cell suspension. During such delivery, port (1.2) is connected to a vacuum source to provide aspiration through the system. Thus, valve port (1.2) is capped with a luer attachment. When solution, such as cell suspension, is drawn into the vessel (1.0) by aspiration, port (1.2) is attached to a vacuum (not shown). Alternatively, port (1.2) may be used for addition of a wash solution, such as saline or buffer, in which case port (1.2) may be connected to a syringe. When drawing up a resuspended solution, such as for reinjection after full processing, port (1.2) is connected to a syringe.

The embodiments shown in Figures 1A and 1B together comprise a tissue processing system in which cooperatively the embodiment shown in Figure 1A represents a collection vessel and the embodiment shown in Figure 1B represents the fractionation vessel. In one embodiment of operation of the system, adipose tissue is collected, optionally washed, and digested in the collection vessel as described above, with the resultant cell suspension collected into an outer chamber of the vessel (100). The external openings of ports (1.1) and (1.2) are compatible with luer locks for ease of connection of transfer components such as a syringe. In the operation of the system of this embodiment, the valve port (1.1) is connected to input port (130) extending into the outer chamber of collection vessel (100). Said connection (not shown) may be via any suitable connection which provides a sealed system through which the cell suspension may travel. Typically the connection is a pharmaceutical or medical grade tubing, typically a ribbed or reinforced tubing capable of maintaining functionality upon the application of a vacuum. Application of a vacuum to the fractionation vessel (1.0) by attachment of a vacuum line, such as tubing described above, to valve port (1.2) permits the cell suspension to be drawn from outer chamber (120) of the collection vessel through port (132) and entering fractionation vessel (1.0) via valve port (1.1), to be deposited in the fractionation vessel through an internal opening located at a lower portion within said fractionation vessel at the end of a U-shaped bend (1.6). Continued processing of the cell suspension may then occur in the fractionation vessel (1.0), by disconnection of the vacuum line from port (1.2) which port is then capped, and disconnection of the collection line (not shown) from valve port (1.1) which is also then capped.

The fractionation vessel (1.0) is thereafter a defined unit separable from the system, within which unit is located the cell suspension. For further processing of the cell suspension the fractionation vessel is centrifuged as described herein to fractionate various components of the cell suspension, typically forming a cell pellet at the base of the vessel (1.0), a free lipid layer above a floating cell layer which comprises adipocytes and an intermediate layer between the cell pellet and the floating cell layer, the intermediate layer being depleted of cells relative to the cell pellet and the floating cell layer. In operation, after centrifugation, the opening (1.6) opens into the intermediate layer between the cell pellet and the floating cell layer. To remove the intermediate layer, the cap, if in place, is removed from port (1.1) to which is then connected a suction, permitting gentle suction to remove the intermediate layer through port (1.6) via tubing (1.5) and exiting the fractionation vessel through port (1.1). A suitable wash solution,

such as saline or buffer may be added to the fractionation vessel (1.0) via either of ports (1.1) or (1.2), the system sealed by capping, inversion or gentle agitation of the vessel to wash the cells and subsequent centrifugation and removal of the intermediate layer between the cell pellet and the floating cell layer by way of opening (1.6) into tube (1.5) and exiting through port (1.1). When processing of the cell suspension is completed, the cell suspension primarily comprises adipose tissue-derived non-adipocyte cells and adipocytes. For removal of the cell suspension from the vessel at the completion of the process, the port (1.2) is uncapped and a syringe connected, the vessel is inverted to permit the cell suspension to collect at the inner surface of the solid covering (1.3), thereby permitting the cell suspension to be drawn out of the vessel through opening (1.4).

Figures 1.1A to 1.1C illustrate usage of the fractionation vessel (1.0) after the addition of the cell suspension. **Figure 1.1A** shows the fat mixture before it has been centrifuged. 1.11 contains the digested fat and saline. **Figure 1.1B** shows the fat sample after it has been centrifuged. 1.12 is the adipocyte layer, 1.13 is the saline and 1.14 is the cell pellet. **Figure 1.1C** shows the sample after the supernatant has been removed. 1.15 is the remaining adipocyte layer and 1.16 is the cell pellet.

Figure 2 is a schematic cross section of one embodiment of a standalone device (2.0) for fat collection and processing, and illustrating in Figure 2.1 the usage of the device. The upper chamber (2.1-2.4) is used to collect the fat and perform the enzymatic digestion. The upper or main chamber may be connected to the lower tube (2.5) through a threaded mechanism (2.4), within which arrangement the digested solution can be filtered through a mesh of determined pore size (2.3). In this embodiment the fabric mesh defines an inner and an outer chamber of the device, the port (2.2) opening into the inner chamber and the port (2.1) opening into the outer chamber. Valve port (2.2) may be capped and is used to deliver the fat into an inner chamber of the pot. Valve port (2.1) is connected to a vacuum output to remove waste from the pot. In the illustrated embodiment filtration device (2.3) is fabric filtration bag of 700 micron pore size, used to suspend the fat whilst it is being washed and drained, the waste draining into the outer chamber.

Threaded attachment (2.4) connects the main chamber to tube (2.5). Tube (2.5) is a centrifuge tube of specified volume. The threaded neck of the tube (2.5) allows it to be connected to (2.4) to facilitate transfer of digested fat (cell suspension) from the main chamber. After filtration, tube (2.5) is spun down in a determined centrifuge, presenting

the cells for further washing, centrifugation as desired, and hence for extraction from the tube as a processed cell suspension such as by pipette.

Figure 3 is a schematic cross section of one embodiment of a standalone device for fat collection and processing. The device comprises two centrifuge tubes connected together at their openings by a threaded plastic connector that contains a mesh filter and a port. When the two centrifuge tubes are connected to the threaded connector their openings are brought together to form a sealed unit comprising of two chambers that are separated by a mesh filter. The Steriflip (Millipore, Billerica, MA, USA) is an example.

In operation, minced adipose tissue or lipoaspirate is placed into a centrifuge tube and collagenase is added. The centrifuge tube is connected to the upper thread on the Steriflip. A second centrifuge tube is connected to the lower thread on the Steriflip. The unit is then incubated to allow digestion of the adipose tissue. The device is then inverted and a vacuum is then applied to the port and the cells are drawn from the first chamber into the second chamber. The Steriflip is then removed from the bottom centrifuge tube and replaced with a cap. The centrifuge tube is then centrifuged to separate the cells from the enzyme.

Figure 4 is a schematic cross section of one embodiment of a device or vessel suitable for collecting and digesting adipose tissue and centrifuging the resultant digested material to provide an adipose tissue-derived cell suspension, optionally comprising adipocytes, according to the methods described herein. The device comprises a sterilizable collection vessel (100) for holding adipose tissue, and for digesting the adipose tissue with a solution comprising proteolytic enzymes, liberated cells, and rinsing solutions. An optional removable lid section (110) allows access to the inside of the collection chamber, for example to allow cleaning if the chamber is to be re-used. A cell permeable screen (120) separating an inner chamber (140) and an outer chamber (150) allows solution comprising cells or small pieces of digested tissue to pass between the inner and outer chambers, whilst retaining large tissue pieces within the inner chamber.

In this embodiment the inner surface of the base of the vessel comprises a conical shape (152), for use of the vessel in centrifugation. In the terminology used elsewhere in this specification this embodiment may be used as a collection and fractionation vessel. The outer surface or shape of the vessel distal to the lid (110) may be conical shaped as shown in Figure 4A. In an alternative embodiment only the inner surface of the base of the vessel (ie., distal to the lid) may comprise a conical shape (152) with the external

surface being of a non-conical shape (Figure 4B). In the illustrated embodiment the (Figure 4B) the external surface of the vessel forms a flat bottom (156) for ease of handling. The flat bottom may be formed through the inclusion of a space between the external surface (156) and the inner conical surface (152), which space may be a void or
5 hollow space or a filled space.

Ports (130) allow the addition and/or removal of tissue, cells and solutions into the inner and/or outer chambers of the collection chamber. Optionally, any one or more of the ports (130) may be adjustable in height allowing them to be lowered or raised inside the vessel. In this embodiment a collection port (132) opens to the outer chamber. The
10 port (132) may be fitted with a tube, which may be detachable, which extends an opening of the port towards the bottom of the vessel, so that liquids lying at the bottom of the vessel may be aspirated. A vacuum line (not shown) may be placed on the collection port if the collection chamber is used to collect adipose tissue during liposuction. The collection port also allows collection of a cell suspension which is generated from
15 enzymic digestion of adipose tissue in the inner chamber.

In this embodiment one port (134) opens to the inner chamber, allowing the addition of adipose tissue, rinsing solution, and enzyme solution into the inner chamber. A delivery port may be connected to a liposuction cannula (not shown) and thus may serve as the port of entry of adipose tissue into the collection chamber during liposuction.

20 The device illustrated in Figure 4 may be used alone in a method of the invention for the collection of adipose tissue, for optional washing of the adipose tissue, for digestion of the adipose tissue, and for centrifugation of the digested material to form an adipose tissue-derived cell suspension, optionally comprising adipocytes.

25

Definitions

Throughout this specification, reference to "a" or "one" element does not exclude the plural, unless context determines otherwise.

In the context of this specification, the term "comprising" means including, but not necessarily solely including. Furthermore, variations of the word "comprising", such as
30 "comprise" and "comprises", have correspondingly varied meanings. Hence, the term "comprising" and variations thereof is used in an inclusive rather than exclusive meaning such that additional integers or features may optionally be present in a composition, method, etc. that is described as comprising integer A, or comprising integer A and B, etc.

In the context of this specification the term "about" will be understood as indicating the usual tolerances that a skilled addressee would associate with the given value.

In the context of this specification, where a range is stated for a parameter it will be understood that the parameter includes all values within the stated range, inclusive of the stated endpoints of the range. For example, a range of "5 to 10" will be understood to include the values 5, 6, 7, 8, 9, and 10 as well as any sub-range within the stated range, such as to include the sub-range of 6 to 10, 7 to 10, 6 to 9, 7 to 9, etc, and inclusive of any value and range between the integers which is reasonable in the context of the range stated, such as 5.5, 6.5, 7.5, 5.5 to 8.5 and 6.5 to 9, etc.

In the context of this specification, the term "plurality" means any number greater than one.

In this specification, the terms "vessel" and "device" are used interchangeably and have the same meaning, except where the context of specific use indicates otherwise.

It is to be noted that reference herein to use of the inventive methods and compositions in treatment or therapy will be understood to be applicable to human and non-human, such as veterinary, applications. Hence it will be understood that, except where otherwise indicated, reference to a patient, subject or individual means a human or a non-human, such as an individual of any species of social, economic or research importance including but not limited to members of the classifications of ovine, bovine, equine, porcine, feline, canine, primates, rodents, especially domesticated members of those classifications, such as sheep, cattle, horses and dogs.

To the extent that it is permitted, all references cited herein are incorporated by reference in their entirety.

25

Detailed Description

The present invention will now be described in more detail, including, by way of illustration only, with respect to the examples which follow.

Provided herein is a method of generating an adipose tissue derived cell suspension from collected adipose tissue, comprising (i) collecting adipose tissue in a collection vessel,

the collection vessel comprising a cell permeable screen which separates an inner chamber and an outer chamber within the collection vessel, at least one port openable from outside the collection vessel into the inner chamber and at least one port openable

from outside the collection vessel into the outer chamber, the adipose tissue being collected into either the inner chamber or the outer chamber of the collection vessel;

(ii) optionally washing the adipose tissue within the collection vessel;

(iii) exposing the adipose tissue to a proteolytic enzyme solution to generate a cell suspension; and

(iv) collecting the adipose tissue cell suspension from the chamber which is other than the chamber in which the adipose tissue was collected.

In a preferred embodiment the step of collecting adipose tissue comprises liposuction.

The cell suspension generated comprises adipocytes and non-adipocyte cells, including mesenchymal stem cells.

The cell suspension may be further processed, for example by centrifugation to remove cells as desired, for example to manipulate the relative proportions of adipocytes and adipose tissue-derived non-adipocyte cells and or to isolate the cells of the cell suspension from the proteolytic enzyme before use, such as reintroduction into subject.

An adipose tissue derived cell suspension comprises viable cells which are suitable for reintroduction into the donor of the adipose tissue. The cell suspension may be used for cell culturing and or for the preparation of adipose tissue-derived cell secretions, such as for therapeutic uses.

Adipose tissue may be human adipose tissue or mammalian animal adipose tissue. The human or animal may be alive or dead, but preferably provided that there are still viable adipocyte cells within the adipose tissue. The adipose tissue may be derived from a mature animal or from a juvenile animal. In particular embodiments the animal is a companion animal, such as a canine or a feline domestic animal, or a working animal. In other particular embodiments the mammal is a farm animal, stud animal, or racing animal such as equines (including horses, donkeys, asses), bovines (including cattle and buffaloes), ovines, caprines, porcines, and camelids (including camels, llamas, alpacas and the like). In other embodiments the animal is a research animal, such as a rodent. In other embodiments the animal is a zoo animal, such as a member of the family Felidae, a member of the family Canidae, a member of the order Rodentia, or a member of the one of the orders of Cetacea, Perissodactyla, Artiodactyla, Tubulidentata, Hyracoidea, Sirenia, or Proboscidea. In preferred embodiments the adipose tissue is bovine or porcine.

In an embodiment the adipose tissue is collected from the same individual to whom the derived cell suspension of the methods of the invention is to be administered. In an embodiment the individual is a human or a non-human mammal having one or more of an

inflammatory condition, a non-inflammatory condition, a bone or cartilage disorder, a liver disorder, a kidney disorder, urinary incontinence, a sporting injury or pain associated with any one or more of the aforesaid conditions or disorders.

Adipose tissue may be collected using known methods. The method described
5 herein is suitable for use on adipose tissue obtained by surgical resection. Where adipose tissue is obtained by surgical resection the adipose tissue is placed into the collection vessel, for example through a sealable port in the collection vessel or, if the collection vessel has a removable and replaceable lid, by opening the lid and placing the adipose tissue within the collection vessel.

10 The adipose tissue may be subjected to mechanical disruption, prior to or after placement in the collection vessel, such as by mincing. Typically, when adipose tissue is obtained by surgical resection, mechanical disruption is performed prior to placement of the tissue in the collection vessel.

The method described herein is suitable for use on adipose tissue obtained by
15 liposuction. Where adipose tissue is obtained by liposuction the liposuction aspirate may be placed in the collection vessel as described for surgically resected adipose tissue. Where adipose tissue is obtained by liposuction the liposuction aspirate may be collected by connecting the collection vessel to the liposuction cannula and to the vacuum source, such that the inside of the collection vessel may be placed under partial vacuum, and the
20 adipose tissue aspirated during liposuction flows under vacuum into the collection vessel where it is collected. Where the collection vessel comprises an inner and an outer chamber, separated by a cell permeable membrane or screen, such as a fabric mesh of defined pore size, the adipose tissue is typically collected into the inner chamber.

The collection vessel may be of any suitable material and of any suitable shape and
25 size. Suitable materials include glass, plastic, resin, metal. The collection vessel may be disposable or may be sterilizable for re-use. A sterilizable vessel may be sterilizable by heat, autoclave, ethylene oxide. The collection vessel may be commercially available, such as the Tissu-Trans® Filtron systems available from Shippert Medical Technologies, Inc. (Centennial, CO 80111, USA). The device may be a device as described herein, such
30 as by reference to the figures and description. The device or vessel may be a device which fits into a centrifuge allowing for the centrifugation of contents of the device or vessel.

A vessel used in the methods of the invention has one or more ports for the introduction or removal of materials in the performance of the invention. Such materials
35 include, for example, lipoaspirate, adipose tissue, liquids such as buffers, water,

proteolytic enzyme solutions and washing fluids, serum, application of vacuum, removal, addition or transfer of cell suspension or parts thereof, such as before or after centrifugation. The ports may be opened or closed (sealed) as desired. The ports may be referred to herein as "openable" ports or as "sealable" ports. When closed, a port may be referred to as "capped". A port may be compatible with attachment of additional components, such as hoses and tubing, syringes, for example through luer lock. Optionally, any one or more of the ports may be adjustable in height allowing it to be lowered or raised inside the vessel. This may, for example, permit the opening of a port inside the vessel to be positioned optimally for the introduction into the vessel or the removal from the vessel of a component. For example, the opening of a port may be adjusted within the vessel to a position suitable for removal from the centrifuged cell suspension of a free lipid layer, or adjusted to a lower position suitable for removal of the intranatant, or adjusted to an intermediate position suitable for the removal of an adipocyte layer. An adjustable port may comprise, for example, alternative lengths of tubing fitted or fittable to the port, an operable wheel or winding mechanism connected or connectable to said port wherein use of the wheel or winding mechanism adjusts the height of the port inside the vessel. A port may comprise a filter or may be compatible with the attachment of a filter, for example an air filter or a liquid filter, to inhibit the passage of biological contaminants into the vessel. A port openable from outside a collection vessel or a fractionation vessel may be created by physical detachment of, for example, the collection vessel from the fractionation vessel. In one such example described herein, the collection vessel and the fractionation vessel may comprise distinct functional regions within a single vessel. When capable of physical separation into separate physical entities by detachment of a connector the detachment may create an opening which can then act as a port for the purposes of the invention. Figure 3 illustrates one such embodiment, whereupon disconnecting the upper and lower parts of the device (as illustrated) creates such an opening. The opening or port can then be used for introduction or removal of materials into or from the device or system. In certain embodiments, at least one of said ports openable from outside the collection vessel or fractionation vessel, respectively, may serve also as a port openable from outside the fractionation vessel or collection vessel, respectively.

The cell permeable screen retains the collected adipose tissue within the collection vessel until a cell suspension has been generated. The cell permeable screen may be of any suitable material and pore size which retains collected adipose tissue prior to digestion and permits passage through of fluids, such as wash solutions, and of cells

released from the digested adipose tissue. Typically, the pore size is about 100 micron to about 900 micron. In preferred embodiments the pore size is between about 400 micron and about 700 micron. More preferably, the pore size is 400 micron. The screen may be anchored to the vessel at any suitable location to permit definition of two compartments within the collection vessel. In certain embodiments in operation according to the method of the invention, adipose tissue is collected into one compartment or chamber of the vessel and, prior to digestion, is restricted from passage to the other compartment or chamber by the cell permeable screen. For example, the cell permeable screen is anchored to the inner sides of the vessel or it may be anchored in the lid of the vessel or it may be anchored in the lid of the vessel. The cell permeable screen may be permanently or detachably anchored to the vessel. For example, where the vessel is of a suitable material for re-use in multiple procedures, typically requiring sterilization between procedures, the cell permeable screen may be detachable for separate cleaning or for disposal and replacement. The cell permeable screen may be substantially rigid within the vessel, such that movement of the screen within in normal operation of the procedure is minimal, or it may be of a flexible material such that it is capable of forming a cell permeable bag-like structure within the vessel.

The adipose tissue after collection may be washed in the device to remove unwanted material. For example, the washing may remove blood and other components which may interfere with further processing or use of the tissue or cells. The washing may utilize any suitable liquid. Typically the washing is done using a sterile saline solution. The liquid used for washing may be heated to a suitable temperature prior to contact with the collected adipose tissue. For example, the liquid may be heated to a temperature in the range of about 35°C to 45°C, preferably to about 37°C, to assist in preparing the adipose tissue for exposure to the proteolytic enzyme solution. Multiple washing steps may be performed, with agitation as desired. In the design of devices described herein for use in the cell processing methods, the washing solution and components removed by the washing drain through the cell permeable screen into a separate compartment or chamber for removal, whilst the adipose tissue is retained for further processing.

The collected, and optionally washed, adipose tissue is digested using a suitable proteolytic enzyme solution. Typically, the enzyme solution comprises collagenase. Collagenase is typically used at a concentration of between about 0.01%w/v and about 0.25%w/v. The enzyme solution may be heated to an appropriate temperature, typically about 37°C, prior to use. Typically, the digestion step includes incubation at a temperature appropriate for the enzyme solution, such as 37°C, and may further include

continuous or occasional agitation or gently shaking, for example by hand or by mechanical device. In one embodiment agitation during digestion comprises stirring the tissue and enzyme solution with the aid of a magnetic stirrer located within the vessel. Digestion continues for an appropriate time to permit the adipose tissue to be digested to a
5 desired degree. During digestion the liquid phase (also now comprising liberated cells) may pass through the cell permeable screen. This may be collected during the digestion, such as continually or intermittently. The so-collected material may be returned to the chamber housing the undigested adipose tissue or may be drawn off for subsequent use. Optionally, the enzyme solution is inactivated on completion of the digestion, such as by
10 addition of serum, preferably autologous serum.

One or more steps of the method of the invention may be conducted in a temperature controlled environment. For example, the temperature controlled environment may permit material within the vessel to be maintained at an appropriate temperature throughout the procedure or throughout one or more steps thereof.
15 Maintenance of the adipose tissue and or cell suspension at about 37°C for example assists in the efficient action of the proteolytic enzyme solution. In an embodiment the temperature controlled environment may comprise housing the vessel in an insulated enclosure with a heat source.

At the completion of a digestion step the adipose tissue may be partially or
20 completely digested. Typically, between about 20% and about 80% of the adipose tissue is digested. Additional proteolytic enzyme may be added if on visual inspection the digestion is deemed insufficient. Similarly, a digestion step may be repeated. It is to be noted, however, that the method of the invention does not require complete digestion of the adipose tissue.

25 The cell permeable screen permits liquid and cells liberated during the digestion to pass from the chamber into which the adipose tissue was collected and into a separate chamber of the device. By reference to terminology used herein, for example, if the adipose tissue is collected into the inner chamber of a device, the cell permeable screen permits liquid and liberated cells to pass to an outer chamber. This material can be
30 referred to as an adipose tissue-derived cell suspension.

When subjected to further processing steps, such as described below, at least some of said further processing is typically performed with the cell suspension housed in a fractionation vessel. A fractionation vessel as used herein is any vessel capable of permitting separation of liquid and cell phases of the cell suspension. Typically, the
35 fractionation vessel is a centrifuge tube or bottle or container. The fractionation vessel

may be a distinct device from the collection vessel, for example such that the two are not typically capable of being connected together to effect transfer. The fractionation vessel may be a device which is capable of being detachably connected to a collection vessel, for example by a physical connection through which said cell suspension may pass, such as a connecting tube, or hose or a cell permeable screen. In certain embodiments, when
5 connected the collection vessel and fractionation vessel may comprise distinct functional regions within a single vessel, an example of which is illustrated in Figure 3.

The fractionation vessel may be the same vessel as the collection vessel such that transfer of the cell suspension is not required prior to centrifugation. In this embodiment
10 the collection vessel is suitable for use in a centrifuge, typically meaning simply that the vessel fits into a centrifuge and so is compatible with use in a centrifuge. A vessel which is suitable for use in a centrifuge may have an inner surface of the base of the vessel formed in a conical shape, for greater ease of use in centrifuging and in identification and or collection of centrifuged material.

The adipose tissue-derived cell suspension may then be further processed as desired. Typically, this further processing comprises centrifugation. Centrifugation of the cell suspension typically results in an intermediate liquid phase (which may be referred to as the intranatant) separating a cell pellet comprising adipose tissue-derived non-adipocyte cells including mesenchymal stem cells from a floating layer of adipocytes. A layer of
20 free lipid above the adipocyte layer may also be present. The intermediate phase is removed and typically discarded, thereby also removing the collagenase. Where present, the layer of free lipid is removed and discarded.

The cell suspension, typically now comprising primarily adipocytes and adipose tissue-derived non-adipocyte cells, may then be subjected to further washing and
25 centrifugation steps as desired. When sufficient washing has been performed to suit a downstream use of the cell suspension, the cells may be collected as desired.

With centrifugation permitting the fractionation of the adipose tissue-derived non-adipocyte cells (pelleted) from the floating layer of adipocytes, the adipocytes and non-adipocyte cells are amenable to separate collection, thereby providing more control over
30 the content of the final cell suspension. It will be appreciated therefore that in any of the centrifugation and removal steps of the method described herein the adipocyte layer may optionally be removed. Where the adipocyte layer is removed the method would produce an adipose tissue-derived cell suspension substantially free of adipocytes. By selective removal and or supplementation, for example, a cell suspension comprising a defined

proportion of adipocytes and of non-adipocyte cells may be formulated. Cells in the cell suspension may be counted.

A cell suspension may be collected at any stage after digestion of the adipose tissue for subsequent use. The cell suspension is suitable for immediate use or may be stored
5 prior to subsequent use, such as at -80°C. Typically, the cell suspension when used for injection into a recipient subject, for example the same subject from which the adipose tissue has been removed, is used without storage or freezing, and typically immediately upon completion of the preparation of the adipose tissue-derived cell suspension. Using
10 the methods described herein an adipose tissue-derived cell suspension, typically comprising adipocytes, may be ready for use, such as for injection into a recipient subject, within a matter of hours, such as less than about one hour, or less than about 1.5 hours, or less than about 2 hours, from the time that the adipose tissue was removed from the subject.

The cell suspension is suitable for various uses, such as for treatment of
15 inflammatory and non-inflammatory conditions, bone and cartilage disorders, liver and kidney disorders, urinary incontinence and sporting injuries, such as are described in Australian Patent Application No. 2009201915 and in International Patent Publication No. WO2010/020005 the entire contents of which are incorporated herein by reference. The cell suspension may be used for cell culturing and or for the preparation of adipose
20 tissue-derived cell secretions, such as for therapeutic uses. The cell suspension may be used for the preparation of pharmaceutical compositions, such as by addition of one or more pharmaceutically acceptable buffers, excipients or carriers.

The invention will now be described in more detail, by way of illustration only,
25 with respect to the following examples. The examples are intended to serve to illustrate this invention and should not be construed as limiting the generality of the disclosure of the description throughout this specification.

Examples

30 Example 1. Processing human adipose tissue to produce a cell suspension containing adipocytes and stromal vascular cells

A sterile plastic container (Figure 1) was used to collect adipose tissue. The tissue could be washed, digested and filtered within the collection vessel (100).

The collection vessel which was used comprised a plastic bowl with a tight fitting lid (110). Three ports (130) were held within the lid. A mesh bag attached to the lid acted as a cell permeable screen (120) and formed an inner chamber (140) and outer chamber (150) within the collection vessel. The mesh of the mesh bag had 700 micron holes.

Human adipose tissue was harvested from the abdomen of a 50 year old female by liposuction using a 3 mm cannula. The cannula was connected by sterile silicon tubing to the sterile collection vessel via a port opening into the inner chamber. A vacuum (0.5 atmospheres) was connected by a vacuum line to collection port which opened into the outer chamber of the collection vessel. A liquid waste trap was connected between the vacuum source and the collection vessel.

While the liposuction was performed the adipose tissue collected within the inner chamber and was retained by the mesh bag, whilst the tumescent fluid was aspirated from the outer chamber of the collection vessel via the collection port to a waste container.

Once approximately 100 grams of adipose tissue was collected in the mesh bag the adipose tissue was washed with saline by inserting the liposuction canula into a 500ml bag of sterile irrigation saline warmed to 37°C. The collection device was tipped onto its side in a way that the collection port opening was at the highest point of the collection vessel, thus maximizing the volume of washing fluid exposed to the adipose tissue within the collection vessel. The vacuum source was disconnected, the vessel turned upright again and rocked to wash the adipose tissue. The suction was then reconnected to drain the washing fluid away.

A 200 ml volume of pre-warmed (37°C) sterile saline plus glucose (0.45% sodium chloride and 2.5% glucose) containing 0.05% collagenase (Sigma Chemical Company) was pipetted into the collection vessel through a delivery port. This allowed the adipose tissue sitting within the inner chamber to be bathed in enzyme solution. The collection vessel was placed in a water bath at 37°C for 1 hour and the contents gently rocked every 10 minutes. A cell suspension was generated from the adipose tissue by action of the enzyme and the cell suspension was able to pass through the mesh bag.

A sterile syringe was connected to the collection port and the cell suspension was drawn into the syringe. A 200ml volume of pre-warmed (37°C) sterile saline was pipetted into a delivery port and the collection vessel was rocked gently. The washings were collected via the collection port and combined with the previously collected cell suspension.

The cell suspension was transferred to 250 ml centrifuge tubes and centrifuged at 1000g at room temp for 10 minutes. The uppermost layer (free lipid) and the layer between the floating adipocytes and the pelleted cells were each removed. The cell pellet and floating adipocytes were combined, 50 ml washing solution (saline/glucose) added
5 and centrifuged again at 1000g for 10 minutes at room temp.

The layer between the pelleted cells and the floating adipocytes was removed, and the combined cell preparation was diluted to a volume of 9 ml with a sterile solution of 0.45% sodium chloride and 2.5% glucose.

10 **Example 2. Collecting cells from digested human adipose tissue as the cells are released from the digested tissue**

Adipose tissue was collected by liposuction using the collection vessel as described in Example 1. The adipose tissue was washed and digested according to Example 1.

During the digestion the cell suspension in the outer chamber was collected at
15 intervals of 20 min via the collection port and centrifuged. The supernatant was returned to the collection vessel and the pellet was resuspended in saline including 10% autologous serum to inactivate the collagenase.

The advantage of this approach is that it allows cells to be harvested and isolated from the proteolytic solution without having to wait for the whole sample to be digested.
20 This improves the viability of the cells within the cell suspension.

Example 3. Generation and use of cells released from digested human adipose tissue for therapy

The purpose of this example was to demonstrate safety of intravenous injection of
25 adipose derived cells generated.

A water bath set at 37°C was used to warm 0.9% NaCl irrigation saline. Human-grade Collagenase (150mg) was dissolved in 10 ml of 0.9% NaCl irrigation saline, and the solution sterilized by passage through a 0.22µm filter.

The external surface of a sterilised collection vessel (Tissu-Trans^R 3-TT Filtron, 500
30 ml, FILTRON Systems) with a 700 micron mesh was wiped with disinfectant and placed in the laminar flow cabinet. The large port of the device was opened and 250 ml of pre warmed irrigation saline was poured in, together with 300 mls of freshly isolated liposuction aspirate from a human subject. The lid was closed and the vessel was shaken.

A sterile suction line was attached to the collection port (VAC) and one of the delivery ports (CAP 1) was slightly opened to avoid negative pressure build up in the collection vessel. The wash fluid was aspirated via the suction line.

5 The previous rinsing steps were repeated twice more so that a total of 750 ml of saline was used for washing. The vacuum line was detached from the collection vessel and all port caps were closed.

300 ml of pre-warmed saline was added to the collection device and 10 ml of filter sterilized pre-prepared sterile collagenase solution was added via a deliver port. The port cap was replaced and the adipose tissue was swirled and shaken gently to immerse all the
10 tissue in the collagenase solution.

The collection vessel was incubated in a water bath at 37°C for 40 minutes, by which time the adipose tissue was digested.

The collection vessel was returned to the laminar flow cabinet and the outer surface disinfected. The digested adipose tissue cell suspension was drawn up into a syringe via a
15 luer lock port in the lid of the collection vessel and transferred to a sterile 200 ml conical centrifuge tube. Additional warmed saline was added to the collection vessel to further wash and release cells trapped in the mesh, this was then collected and added to the cell suspension. This rinse was repeated.

The cell suspension was centrifuged at 1000g for 10 minutes. The entire lipid layer,
20 adipocytes cell layer and saline layer were removed, leaving a non-adipocyte cell pellet. The cell pellets were re-suspended and again centrifuged at 1000g for 10 minutes, and the lipid, adipocyte and saline layers were removed.

The cell suspension was re-suspended in a final volume of 8 ml. 0.5 ml was kept for cell analysis and 7.5 ml was administered to the subject.

25 The total cell processing time from commencement was 2 hours and 25 minutes. Approximately 1.3×10^8 cells were obtained with approximately 66% of the cells viable.

The cell suspension was injected slowly in 0.5 ml doses intravenously into the patient under close supervision. The patient was suffering from motor neuron disease.

The subject experienced a mild reaction with first 2 ml dose, including numb face,
30 tingling, mild increase in heart rate and fall in blood pressure. There was no change in the saturation of oxygen in the blood. The subject experienced a throbbing holocephalic headache which lasted for five minutes, and which then settled without intervention.

After one day the subject's vital signs were normal.

Example 4: Alternative protocol for clinical application**(i) Adipose Tissue Harvest protocol**

In this embodiment a 3-TT-Filtron 500 ("fat trap"; Shippert Medical Technologies, Inc., Centennial, CO 80111, USA) is used as the device into which the adipose tissue is collected and processed to produce an initial cell suspension from the adipose tissue. Disconnect the existing tubing from the *PATIENT* port, close all ports with the attached lids and attach the fat trap to the side of the aspirator. Attach the side with the outer PVC tube reinforcement to the *VAC* port of the fat trap and the other end to the waste container.

Turn on aspirator and set liposuction vacuum to no more than -15 inHg (equivalent to -380 mmHg, -50 kPa, -0.5 bar). Turn aspirator off, connect the canula (3-4mm Mercedes tip canula) to the *PATIENT* port using the ribbed standard suction tubing and perform liposuction until sufficient fat is obtained.

(ii) Adipose tissue processing protocol

Under sterile conditions collected fat is washed by addition of approximately 250 mL of sterile saline. Wash solution is removed to waste by vacuum. This is designed to wash the fat granules retained in the sieve from debris and blood. The washing step may be repeated as desired.

Digestion of the adipose tissue is commenced by addition of (GMP grade) collagenase to a final concentration of approximately 0.05%w/v for human adipose tissue and incubation at 37°C for approximately 30 minutes with occasional gentle shaking.

The digested fat is drawn through the *VAC* port by syringe and transferred to a suitable tube. Pre-warmed normal saline is added to the fat trap through the *CAP1* port, and, after agitation, the contents are again collected by syringe as described. Once all digested material has been removed from the fat trap the collected material is centrifuged at 1000 x g (1970 RPM) for 5 min (using "low" break setting).

After centrifugation the intermediate layer is removed by aspiration, thereby also removing collagenase, being careful to keep the adipocytes and not to disturb the pellet. Additional washing of the cells is then performed using sterile saline solution, gentle mixing and centrifugation as described above, each time removing at least the intermediate liquid phase after centrifugation.

Additional washing steps may be performed as desired on the cell suspension. During, or at the completion of, washing, cells are collected and retained as desired. This may permit, for example, the production of a cell suspension which has relative

- proportions of adipocytes to non-adipocytes which reflects that of the starting material, although allowing for loss of cells during the processing, or which may be different by design to that found in the starting material, such as a cell suspension enriched in adipocytes relative to adipose tissue-derived non-adipocyte cells or enriched in adipose
- 5 tissue-derived non-adipocyte cells relative to adipocytes.

Claims

1. A method of generating an adipose tissue derived cell suspension from collected adipose tissue, comprising:
 - 5 (i) collecting adipose tissue in a collection vessel,
the collection vessel comprising a cell permeable screen which separates an inner chamber and an outer chamber within the collection vessel, at least one port openable from outside the collection vessel into the inner chamber and at least one port openable from outside the collection vessel into the outer chamber, the adipose tissue being
10 collected into either the inner chamber or the outer chamber of the collection vessel;
 - (ii) optionally washing the adipose tissue within the collection vessel;
 - (iii) exposing the adipose tissue to a proteolytic enzyme solution to generate a cell suspension; and
 - (iv) collecting the adipose tissue cell suspension from the chamber which is
15 other than the chamber in which the adipose tissue was collected.
2. The method according to claim 1, wherein the vessel is suitable for use in a centrifuge.
3. The method according to claim 2, wherein the method further comprises,
prior to said collecting in step (iv), centrifuging said vessel to fractionate components of
20 said cell suspension to form a cell pellet, a cell layer which comprises adipocytes, an intermediate layer which is depleted of cells relative to the cell pellet and the adipocyte layer, and, where present, a free lipid layer; and removing the intermediate layer and, where present, the free lipid layer through a port openable from outside the collection vessel, thereby retaining within said vessel an adipose tissue-derived cell suspension
25 comprising adipocytes.
4. The method according to claim 3, wherein said removing is through a port openable from outside the collection vessel into the chamber into which the adipose tissue was collected.
5. The method according to claim 3, wherein the method further comprises,
30 after said centrifugation and removing, washing the remaining cell suspension by addition of a suitable liquid and repeating said centrifugation and said removing.
6. The method according to claim 1, wherein the method further comprises, after said collecting in step (iv), transferring said collected adipose tissue cell suspension to a fractionation vessel and centrifuging said vessel to form a cell pellet, a cell layer

which comprises adipocytes, an intermediate layer which is depleted of cells relative to the cell pellet and the adipocyte layer, and, where present, a free lipid layer; and removing the intermediate layer and, where present, the free lipid layer; thereby retaining within said fractionation vessel an adipose tissue-derived cell suspension comprising adipocytes.

5 7. The method according to any one of claims 1 to 6, wherein one or more of a liquid for washing the adipose tissue and the proteolytic enzyme solution is warmed to 37°C prior to contact with said adipose tissue.

 8. The method according to any one of claims 1 to 7, wherein the step of collecting adipose tissue comprises liposuction.

10 9. The method according to any one of claims 1 to 7, wherein the proteolytic enzyme solution in the exposing step comprises collagenase at a concentration between about 0.01%w/v and about 0.25%w/v.

 10. An adipose tissue derived cell suspension comprising adipocytes prepared according to the method of any one of claims 1 to 9.

15 11. An adipose tissue-derived cell suspension according to claim 10, for use in the preparation of a pharmaceutical composition.

 12. A tissue processing system comprising:

- a collection vessel comprising a cell permeable screen which separates an inner chamber and an outer chamber within the collection vessel, at least one port openable from outside the collection vessel into the inner chamber and at least one port openable from outside the collection vessel into the outer chamber, and
- a fractionation vessel comprising at least two ports openable from outside the fractionation vessel to inside, whereby in use at least one of said ports is detachably connected to at least one port of said collection vessel.

25 13. The tissue processing system according to claim 12, wherein said fractionation vessel comprises a solid covering over the vessel, through which said at least two openable ports are located.

 14. The tissue processing system according to claim 12, wherein an internal opening of at least one of said openable ports is positioned at a lower portion within the fractionation vessel.

30 15. The tissue processing system according to claim 14, wherein said internal opening is located at a lower portion within said fractionation vessel at the end of a U-shaped bend, wherein the attitude of the opening is away from the bottom of the vessel.

16. The tissue processing system according to claim 12, wherein said collection vessel and said fractionation vessel are detachably connected to form a single vessel.

17. The use of a tissue processing system according to any one of claims 12 to 5 16 for the preparation of an adipose tissue-derived cell suspension.

18. A tissue processing vessel comprising a cell permeable screen which separates an inner chamber and an outer chamber within the vessel, at least one port openable from outside the vessel into the inner chamber and at least one port openable from outside the vessel into the outer chamber, wherein the vessel is suitable for use in a 10 centrifuge.

19. The tissue processing vessel according to claim 18, wherein an inner surface of said vessel comprises a conical shape suitable for centrifugation of a cell suspension within said vessel.

20. The tissue processing vessel according to claim 18 or 19, wherein the outer 15 base of the vessel is flat.

21. The tissue processing vessel according to any one of claims 18 to 20, wherein the vessel comprises two ports openable from outside the vessel into the inner chamber or comprises three ports openable from outside the vessel into the inner chamber.

22. The tissue processing vessel according to any one of claims 18 to 21, 20 wherein the vessel comprises two ports openable from outside the vessel into the outer chamber or comprises three ports openable from outside the vessel into the outer chamber.

23. The tissue processing vessel according to any one of claims 18 to 22, wherein one or more of said ports comprises an opening within the region of the vessel defined by the conical shape.

24. The tissue processing vessel according to any one of claims 18 to 23, 25 wherein one or more of said ports comprises an opening within said vessel suitable for the removal of an intermediate layer which is depleted of cells relative to a cell pellet and a floating adipocyte layer following centrifugation of an adipose tissue cell suspension.

25. The tissue processing vessel according to any one of claims 18 to 24, 30 wherein one or more of said ports comprises an opening within said vessel suitable for the removal of a free lipid layer following centrifugation of an adipose tissue cell suspension.

26. The tissue processing vessel according to any one of claims 18 to 25, wherein an opening of one or more of said ports within said vessel is comprised in a detachable tube fitted to said port.

27. The tissue processing vessel according to any one of claims 18 to 26, wherein one or more of the ports is adjustable in height and can be lowered or raised inside the vessel.

28. The use of a tissue processing vessel according to any one of claims 18 to 27 for the preparation of an adipose tissue-derived cell suspension.

Figure 1

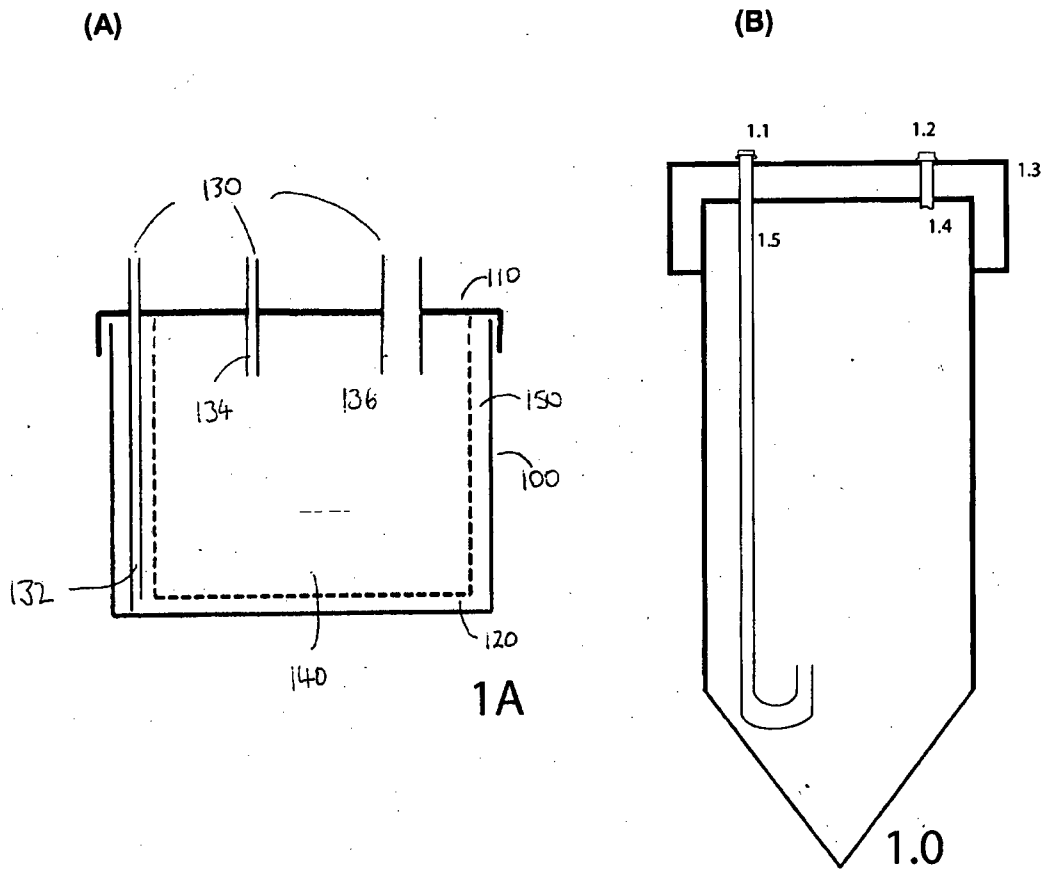


Figure 1.1A

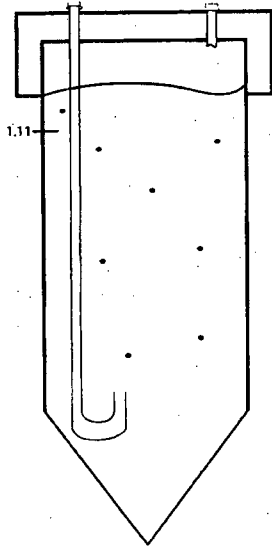


Figure 1.1B

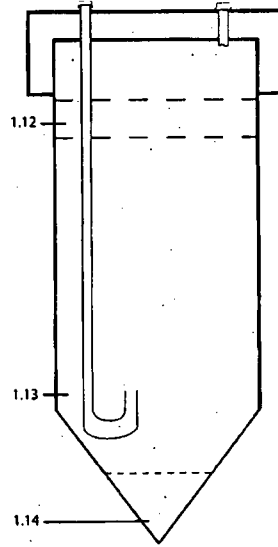


Figure 1.1C

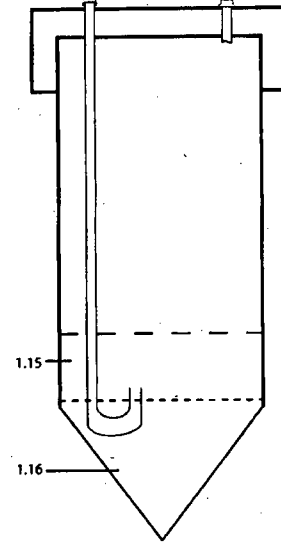


Figure 2A

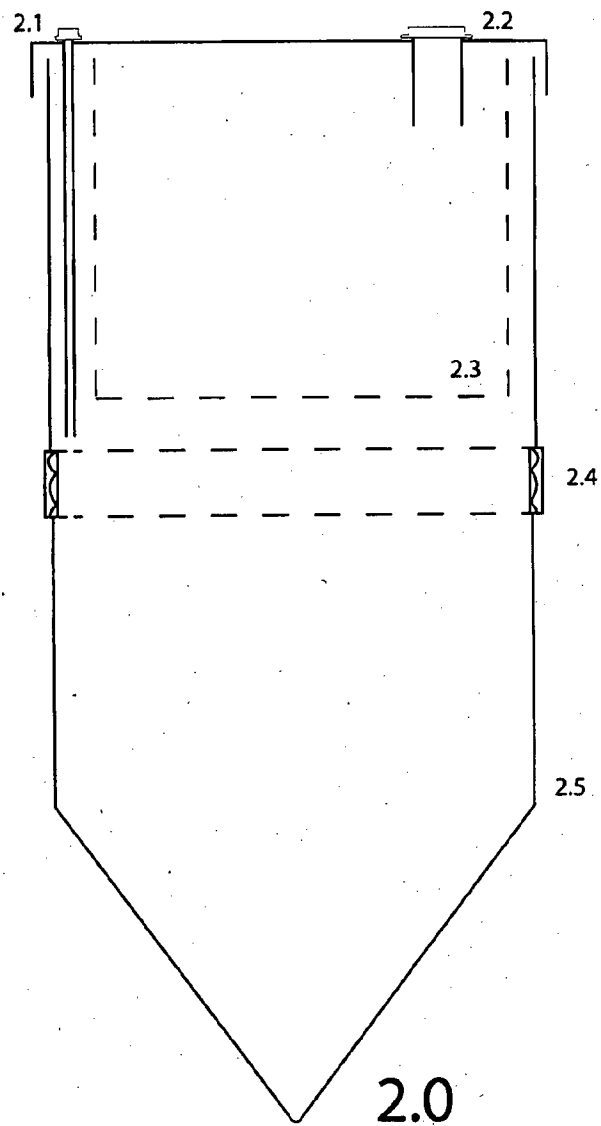


Figure 2B

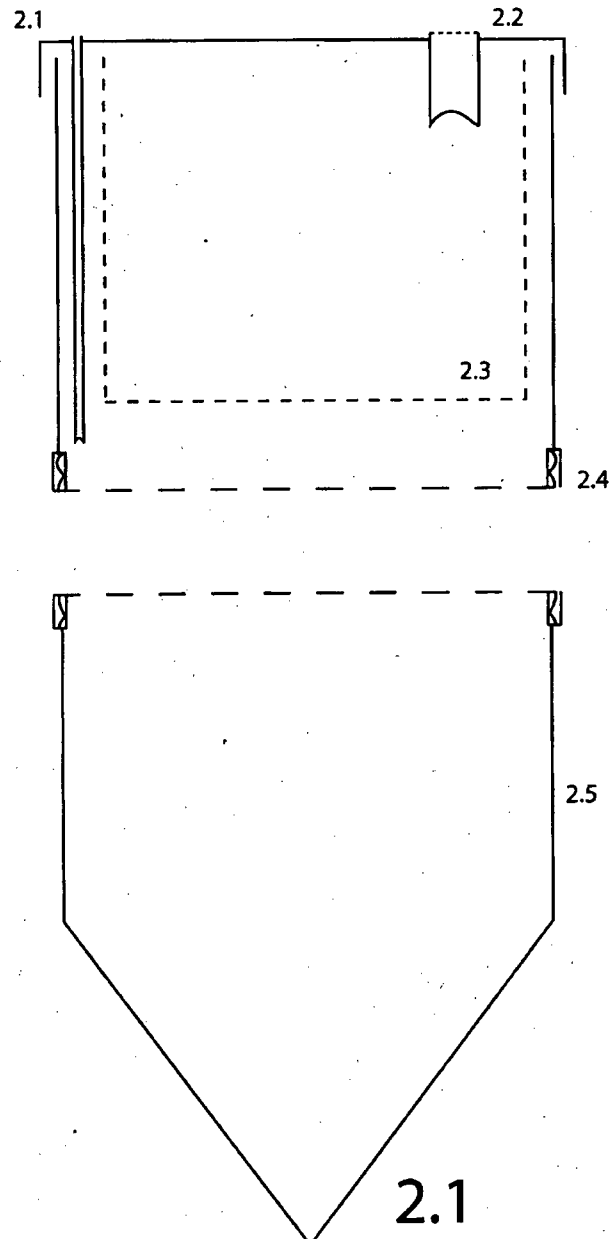
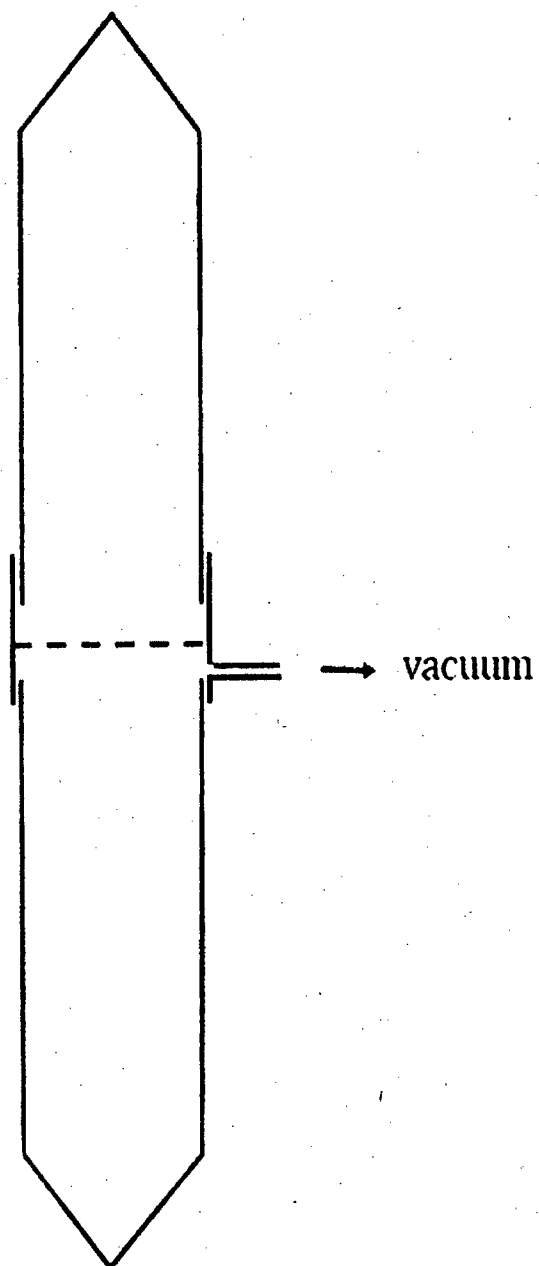


Figure 3

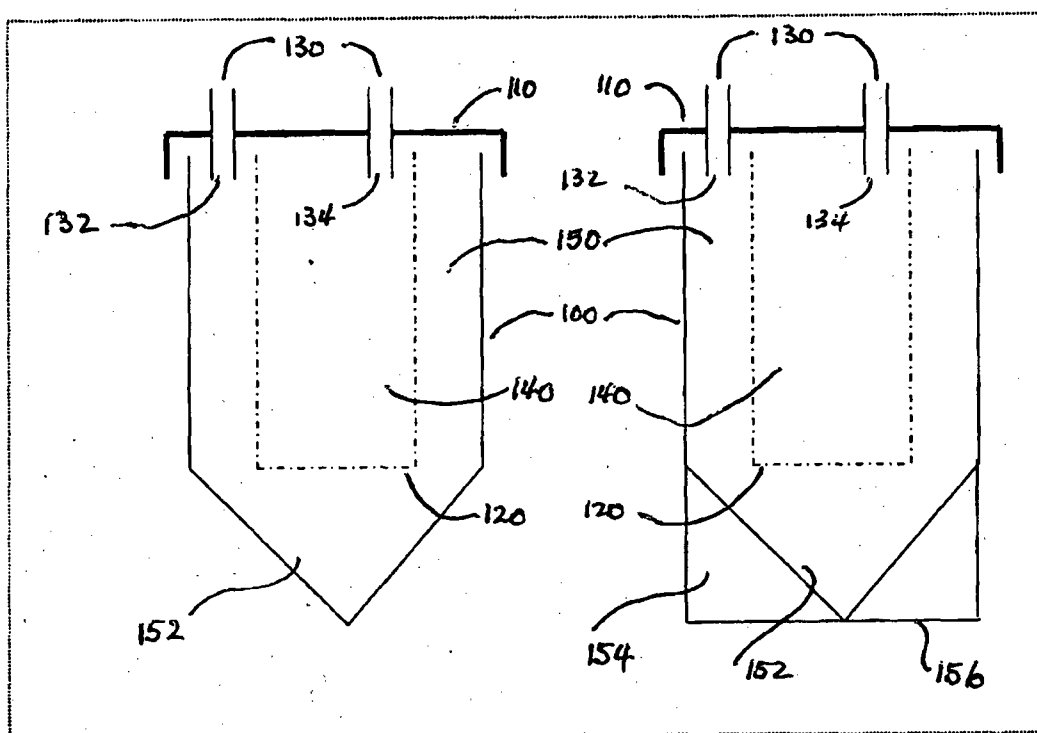


Figure 4A

Figure 4B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2012/000272

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

C12N 5/077 (2010.01)

C12M 3/08 (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPODOC, WPI, MEDLINE, HCAPLUS, BIOSIS, EMBASE, BIOTECHABS: cell suspension, adipose, filter, proteolysis, fractionate and like terms

Google: Regeneus; Espacenet: Applicant: Regeneus Inventor: Ben Herbert, Graham Vesey, Richard Lilischkis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0984060 A2 (BAXTER INTERNATIONAL INC.) 8 March 2000 See the whole document, particularly the Figures, [0030]-[0035] and [0038]	1-28
X	WO 2005/095581 A1 (MACROPOROUS BIOSURGERY INC.) 13 October 2005 See the whole document, particularly Figures 1-5 and 7-8, pages 12-32 and page 70 lines 34-35	1-28
X	WO 2010/129817 A1 (TISSUE GENESIS, INC.) 11 November 2010 See the whole document, particularly Figure 3 and page 15 line 31 to page 20 line 8	1-28
X	US 5,786,207 A (Katz et al.) 28 July 1998 See the whole document, particularly Figures 1-4, columns 3-14 and the claims	1-28

☒ Further documents are listed in the continuation of Box C☒ See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search
30 March 2012Date of mailing of the international search report
2 April 2012

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2012/000272

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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X	US 2010/0285588 A1 (Stubbers et al.) 11 November 2010 See the whole document, particularly the abstract, Figure 6, [0073], [0076], [0080]-[0087] and [0103]	1-28
P, X	US 2011/0111497 A1 (TAMAI et al.) 12 May 2011 See the whole document, particularly the abstract, Figure 1 and [0055]-[0091]	1-28
P, X	US 2011/0117650 A1 (Riordan) 19 May 2011 See the whole document, particularly the Figures and [0024]-[0045]	1-28

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2012/000272

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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END OF ANNEX							