(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 October 2015 (22.10.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/160952 A1

(51) International Patent Classification:
A61K 9/00 (2006.01) *C12N 5/0797* (2010.01)
A61K 48/00 (2006.01) *C12N 5/071* (2010.01)

(21) International Application Number:
PCT/US2015/025973

(22) International Filing Date:
15 April 2015 (15.04.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/980,015 15 April 2014 (15.04.2014) US
61/987,150 1 May 2014 (01.05.2014) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,

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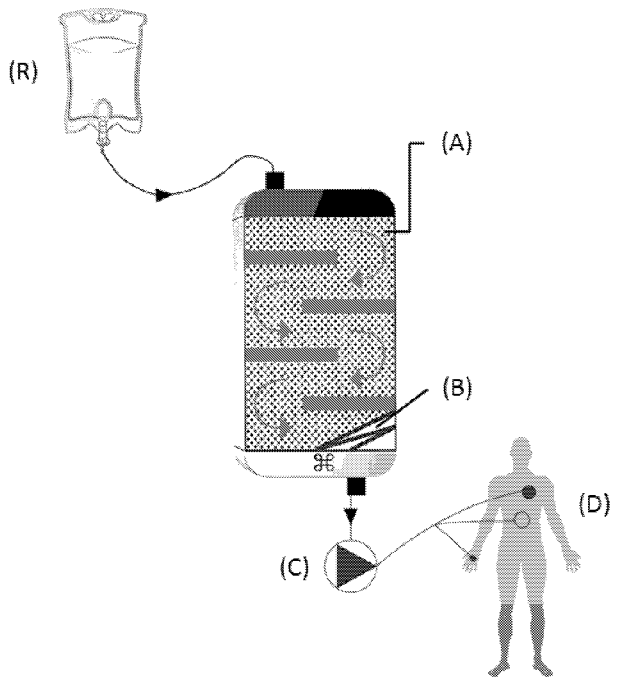
(54) Title: APPARATUS OR DEVICE FOR CELLULAR THERAPY

(57) Abstract: The invention features an apparatus or device for implanted, *ex vivo*, or extracorporeal cell therapy, methods of using the apparatus or device to treat various medical conditions, such as an autoimmune disease, neurodegenerative disease, cancer, inflammatory conditions, cardiac infarction, chronic heart disease, acute respiratory distress, trauma, and organ failure, and a kit containing the apparatus or device. The apparatus or device can be connected to a subject, organ, or tissue, and can be used to transfer therapeutic substances produced by immortal cells that remain resident in the apparatus or device to the subject, organ, or tissue.

FIG. 7



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LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

APPARATUS OR DEVICE FOR CELLULAR THERAPY

BACKGROUND

Cellular therapy using human mesenchymal stem cells (hMSCs) has advanced to clinical trials and the first products have been approved in Canada, Switzerland, Korea, and New Zealand for graft vs host disease (GvHD) (Cyranoski, *Nature Biotechnology* 30:571, 2012). While approval for such products is pending in the U.S. and many other countries, compassionate use is allowed in the U.S. and several countries. The first human infusions with MSCs were performed over 20 years ago at Case Western University/University Hospital (Lazarus et al., *Bone Marrow Transplant* 16:557-64, 1995). Since then, over 7,000 patients have received MSCs in phase I, II, and III clinical trials and there are currently over 380 trials including over 180 open trials utilizing MSCs. The safety profile of infused human MSCs is surprisingly strong, both at early time points of infusion and longer term follow-up periods (Lalu et al., *PLoS One* 7:1-21, 2012; Leistner et al., *Clin. Res. Cardiol.* 100:925-34, 2011). MSCs have been directly injected into organs (heart, muscle, kidney), joints for osteoarthritis, and infused systemically for support of hematopoietic transplant, therapeutic treatment of autoimmune diseases, GvHD, and to modify the course of disease after organ damage such as cardiac infarction, chronic heart disease, and acute respiratory distress.

The hMSCs produce many soluble bioactive factors that may be responsible for their broad application (Haynesworth et al., *J. Cell. Physiol.* 166:585-92, 1996; Majumdar et al., *J. Cell. Phys.* 176:57-66, 1998; Pittenger et al., *Science* 284:143-147, 1999; Aggarwal and Pittenger, *Blood* 105:1815-1822, 2005; Klyushnenkova et al., *J. Biomedical Sciences* 12:47-57, 2005; Di Nicola et al., *Blood* 99:3838-43, 2002). Because of the many therapeutic uses for hMSCs, there is strong interest in understanding the therapeutic capabilities of hMSCs and finding standardized methods for their safe and effective use. However, due to differences in the source of hMSCs and lab procedures, there is limited agreement on the identity, purity, potency, or recommended dose of such cells. Furthermore, delivery of hMSCs by systemic infusion or needle injection to solid organs results in loss of control and absence of knowledge of where the cells are or what they are doing in the body after infusion. Also, there is no method to remove the delivered cells from the body (other than transduction with a suicide gene).

We have used in vivo imaging technology to follow delivered MSCs but this technology only gave very limited information about the delivered cells and did not provide actual manipulation of the cells once they are inside the body (Kraitchman et al., *Circulation* 112:1451-61, 2005; Walczak et al., *Stroke* 39:1569-74, 2008). More importantly, the survival of MSCs (and other stem cells) after systemic delivery or needle injection to the target tissue is very limited. An estimate of the percentage of MSCs surviving longer than 5 days is about 1-2%. It is unclear why this is so, but it is partially due to the adaptation of the cultured MSCs to *in vitro* conditions, the shock of re-entry into the body, and the difficulty in finding a receptive niche *in vivo*.

Current cell therapy using hMSCs is impeded because the infusion or local injection of hMSCs leads to a loss of control of the therapeutic dose, and the disposition, location, and action of the cells in the body can only be inferred. Moreover, cell therapy requires time consuming steps that must be performed under current Good Manufacturing Practices (cGMP) that are standard in the industrial and

therapeutic manufacturing fields. These practices add significant time and cost to cellular therapies. There exists a need for standardized cells to be used in cellular therapy and for reproducible methods for delivering the "cellular therapeutic," which can greatly decrease costs and accelerate the adoption of such therapies. Therefore, novel cellular therapies are needed.

SUMMARY OF THE INVENTION

The invention features a cell therapy apparatus or device containing an immortalized cell (e.g., immortalized stem or progenitor cells) that can be connected to the blood circulation system of an organ, tissue, or recipient. The immortalized stem or progenitor cells produce therapeutic substances, such as cytokines, chemokines, anti-inflammatory agents, and exosomes, that can be infused to repair a damaged or deficient organ, tissue, or cell (e.g., lung, heart, kidney, liver, and other organs, tissue, or cells). The cell therapy apparatus or device can be used to treat focused tissue damage of an organ, tissue, or recipient, or diffused disease or tissue damage such as sepsis or graft vs host disease. The cell therapy apparatus or device retains the immortalized stem or progenitor cells and only transfers the therapeutic substances produced by the immortalized stem or progenitor cells to the organs, tissues, or cells. The apparatus or device contains a porous material (e.g., a matrix) on which the cells reside. The volume of the apparatus or device is such that it can contain a therapeutically useful number of cells (e.g., in the range of about 50,000 to about 200 million (e.g., about 500,000 to about 200 million)). The apparatus or device may contain any kind of cell with useful properties; particularly useful are immortalized cells, such as immortalized stem or progenitor cells.

In one aspect, the invention features an apparatus or device containing an inlet port and an outlet port for connection to an organ, tissue, or subject, in which the apparatus or device further contains immortalized cells resident on a matrix in the apparatus or device. In an embodiment, the apparatus or device is adapted for connection to the blood circulation system of an organ, tissue, or recipient. In other embodiments, the apparatus or device includes two or more inlet ports or two or more outlet ports.

In some embodiments, the apparatus or device is for *ex vivo* use or implanted in the subject.

In other embodiments, the immortalized cells in the apparatus or device are stem or progenitor cells.

In some embodiments, the cells in the apparatus or device produce effluent medium, wherein said effluent medium is conditioned by the cells.

In some embodiments, the immortalized cells in the apparatus or device are cells of the lung, heart, liver, bladder, brain, nervous system tissue, blood vessels, skin, eye structures, gut, bone, muscle, ligament, cartilage, esophagus, pancreas, intestines, gallbladder, bile duct, fallopian tubes, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, ureter, urethra, uterus, or fat.

In some embodiments, the stem or progenitor cells are embryonic stem cells, induced pluripotent stem cells (IPS), hematopoietic stem cells, intestinal stem cells, osteoblastic stem cells, mesenchymal stem cells (MSCs), multipotent adult progenitor cells (MAPCs), neural stem cells, epithelial stem cells, bone stem cells, cardiac myocyte progenitor stem cells, skin stem cells, skeletal stem cells, muscle stem cells, endothelial stem cells, endothelial progenitor cells, umbilical cord stem cells, adipose stem cells, placental stem cells, placental-derived multipotent stem cells, or liver stem cells.

In particular, the mesenchymal stem cells are selected from the group consisting of lung mesenchymal stem cells, Wharton's Jelly mesenchymal stem cells, bone marrow mesenchymal cells, bone marrow stromal cells, umbilical cord mesenchymal cells, spleen mesenchymal cells (e.g., $Hox11^+$, $CD45^-$ cells), adipose derived mesenchymal cells, and pericytes. The epithelial stem cells are selected from the group consisting of lung epithelial stem cells, breast epithelial stem cells, vascular epithelial stem cells, and intestinal epithelial stem cells. The skin stem cells are selected from the group consisting of epidermal stem cells, follicular stem cells, and follicle bulge stem cells. The neural cells are selected from the group consisting of neuronal dopaminergic stem cells and motor neuron stem cells.

In some embodiments, the stem cells in the apparatus or device are mesenchymal stem cells (MSCs).

In some embodiments, the inlet port or outlet port of the apparatus or device has one or more of a pre-filter, intermediate filter, or final filter.

In some embodiments, one or more of the pre-filter, intermediate filter, and final filter of the inlet port or outlet port has a filter size in the range of 0.2 – 1 μm. In particular, the pre-filter, intermediate filter, and final filter have a filter size of 1 μm, 0.48 μm, and 0.22 μm, respectively.

In some embodiments, the matrix of the apparatus or device includes polyvinyl acetal, polylactic acid (PLA), polyglycolic acid (PGA), poly(lactic/glycolic acid) (PGLA), hollow fiber substrate, titanium, MATRIGEL® (e.g., reconstituted basement membrane extract that is rich in one or more of laminin, growth factors, entactin/nidogen, type I, II, III, IV, or V collagen, and heparan sulfate proteoglycan (perlecan)), fibronectin, or gelatin.

In some embodiments, the polyvinyl acetal, polylactic acid, or polyglycolic acid is a foam or woven or nonwoven fabric material.

In some embodiments, the apparatus or device is constructed from polycarbonate, polyacrylate, polystyrene, polysulfone, polyester, poly(methyl methacrylate) (PMMA), polymethacrylate (PMA), poly tetrafluoroethylene (PTFE), or fluorinated ethylene propylene copolymer (FEP).

In particular, the apparatus or device is constructed from polycarbonate or fluorinated ethylene propylene copolymer (FEP).

In some embodiments, the apparatus or device is constructed of flat sheets of material and fused along the edges to create a bag. In some embodiments, the bag contains a matrix.

In other embodiments, the apparatus or device includes a straight, U-shaped, Z-shaped, or multichannel fluid flow path. In particular, the apparatus or device utilizes the Z-shaped fluid flow path.

In some embodiments, the apparatus or device has a length of between 1 to 5 inches (e.g., 2 to 5 inches), a width of between 1 to 10 inches (e.g., 3 to 10 inches), and a thickness of between 0.2 to 1.5 inches; particularly the apparatus or device is approximately 3 inches by 5 inches by 0.75 inches.

In other embodiments, the apparatus or device includes a volume of approximately 1 to 100 cm^3 (e.g., 5 to 100 cm^3).

In other embodiments, the apparatus or device is constructed of a hard or resilient material. In alternative embodiments, the apparatus or device is constructed of a soft or flexible material. In other embodiments, the apparatus or device includes a tube (e.g., a catheter) connected to the one or more inlet ports or the one or more outlet ports. The tube can be made from, e.g., latex, silicone, polyvinylchloride, polyethylene, or polytetrafluoroethylene (TEFLON®). In other embodiments, the

catheter is a Foley catheter, straight catheter, or Quinton catheter. The tube may also include an adapter for attaching the tube to other devices.

In some embodiments, the apparatus or device further includes an appropriate cell culture medium for the cells. In some embodiments, the cell culture medium is selected from phosphate-buffered saline (PBS), Dulbecco's Modified Eagle Medium (DMEM), F-12 medium, α Modified Eagles Medium (α-MEM), or any one or a mixture of these media supplemented with fetal bovine serum (FBS) or human serum, blood, or one or more blood products such as platelet-rich plasma, serum albumin, or recombinant serum albumin. In particular, the cell culture medium in the apparatus or device is PBS.

In other embodiments, the apparatus or device further includes a heating unit that maintains the immortalized cells in the apparatus or device at a temperature in the range of about 20-37°C (e.g., 30-37°C).

In some embodiments, the apparatus or device is connected to an organ or tissue of a subject or to the circulatory system of the subject. In particular, the organ or tissue is *in vivo* or *ex vivo*.

In other embodiments, the apparatus or device is connected to the circulatory system of the subject.

In some embodiments, the apparatus or device is connected extracorporeally to the subject.

In some embodiments, the apparatus or device is implanted into the subject.

In other embodiments, the apparatus or device is connected immediately proximal to the organ or tissue.

In some embodiments, the subject is a transplant recipient, and the apparatus or device is connected to the subject in preparation for transplantation of an organ or tissue.

In other embodiments, the organ or tissue connected to the apparatus or device is being prepared for transplantation into a recipient.

In some embodiments, the apparatus or device including immortalized cells is infused with a bioactive factor or a cell that causes the immortalized cells (e.g., the resident, immortalized cells) in the apparatus or device to produce one or more molecules not otherwise made in the apparatus or device. In some embodiments, the bioactive factor is TGFβ or TGFα. In some embodiments, the cell that causes the immortalized cells in the apparatus or device to produce one or more molecules not otherwise made in the apparatus or device is a peripheral blood mononuclear cell

In some embodiments, the immortalized cells in the apparatus or device are bioengineered to produce one or more molecules. In some embodiments, the immortalized cells in the apparatus or device are bioengineered to produce insulin.

In some embodiments, the apparatus or device includes a biological sample from a donor or a recipient. The biological sample may include one or more cells, fluid, or bioactive factors obtained from the donor or the recipient. In particular, the biological sample is injected into the apparatus or device.

In some embodiments, the organ or tissue connected to the apparatus or device is from lung, heart, liver, bladder, brain, blood vessels, skin, eye structures, gut, bone, muscle, ligament, cartilage, esophagus, pancreas, intestines, gallbladder, bile duct, fallopian tubes, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, ureter, urethra, or uterus.

In other embodiments, the apparatus or device is connected to a container that includes stem or progenitor cells isolated from a subject.

In all embodiments of the first aspect of the invention, the subject or recipient is a human or a non-human mammal. Particularly, the subject or recipient is a human. In other embodiments, the organ or tissue to which the apparatus or device is connected is a human organ or tissue.

In other embodiments, the apparatus or device promotes proliferation of the stem or progenitor cells. The apparatus or device can be used to promote proliferation of stem or progenitor cells, such as hematopoietic stem cells, inside the device, or outside the device (e.g., *in vitro* or *in vivo*).

In yet other embodiments, the immortalized cells in the apparatus or device are human cells, particularly the human cells are human stem or progenitor cells, such as human MSCs (e.g., human bone marrow MSCs).

A second aspect of the invention features a method of treating a medical condition by establishing a fluid communication between the apparatus or device of the first aspect of the invention and an organ, tissue or subject. The subject is a human or a non-human mammal; particularly, the subject is a human. In other embodiments, the organ or tissue is a human organ or tissue.

In other embodiments, the method uses immortalized cells, such as human cells; particularly the human cells are human stem or progenitor cells, such as human MSCs (e.g., human bone marrow MSCs).

In other embodiments of the method, the organ or tissue is in a subject or is *ex vivo*; particularly the organ or tissue is in the subject.

A third aspect of the invention features a method of treating a medical condition in a subject including collecting the effluent medium produced by the cells in the apparatus or device of the invention and administering the effluent medium to the subject, wherein the effluent medium is conditioned by the cells.

A fourth aspect of the invention features a method for analyzing and identifying for molecules in the effluent medium produced by the cells in the apparatus or device of in the invention including collecting the effluent medium, detecting in the effluent medium one or more molecules produced by the cells, and identifying and isolating the one or more molecules produced by the cells, wherein the effluent medium is conditioned by the cells.

In some embodiments, the methods further include infusing the apparatus or device of the invention with a bioactive factor or a cell that causes the immortalized cells in the apparatus or device to produce one or more molecules not otherwise made in the apparatus or device. In some embodiments, the bioactive factor is TGFβ or TGFα. In some embodiments, the cell that causes the immortalized cells in the apparatus or device to produce one or more molecules not otherwise made in the apparatus or device is a peripheral blood mononuclear cell

In some embodiments, the method is used to treat a medical condition. The medical condition is an acute disease, a chronic disease, an autoimmune disease, a skin disease, a neurodegenerative disease, a musculoskeletal disease, a muscle wasting disease, cancer, a vascular or circulatory disease, an inflammatory condition, a cytokine storm condition, an immunological condition, graft versus host disease, a rheumatology disease, cardiac infarction, acute or chronic heart disease, acute or chronic respiratory disease or distress, pulmonary fibrosis, trauma, sepsis, or organ failure.

In particular, the autoimmune disease is Alopecia Areata, Ankylosing Spondylitis, Antiphospholipid Syndrome, Addison's Disease, Hemolytic Anemia, Hepatitis, Behcets Disease, Bullous

Pemphigoid, Cardiomyopathy, Celiac Sprue-Dermatitis, Chronic Fatigue Immune Dysfunction Syndrome (CFIDS), Chronic Inflammatory Demyelinating Polyneuropathy, Churg-Strauss Syndrome, Cicatricial Pemphigoid, Limited Scleroderma (CREST Syndrome), Cold Agglutinin Disease, Crohn's Disease, Discoid Lupus, Essential Mixed Cryoglobulinemia, Fibromyalgia-Fibromyositis, Graves' Disease, Guillain-Barré Syndrome, Hashimoto's Thyroiditis, Hypothyroidism, Idiopathic Pulmonary Fibrosis, Idiopathic Thrombocytopenia Purpura (ITP), IgA Nephropathy, insulin dependent diabetes, Juvenile Arthritis, Lichen Planus, Lupus, Ménière's Disease, Mixed Connective Tissue Disease, Multiple Sclerosis, Myasthenia Gravis, Pemphigus Vulgaris, Pernicious Anemia, Polyarteritis Nodosa, Polychondritis, Polyglandular Syndromes, Polymyalgia Rheumatica, Polymyositis and Dermatomyositis, Primary Agammaglobulinemia, Primary Biliary Cirrhosis, Psoriasis, Raynaud's Phenomenon, Reiter's Syndrome, Rheumatic Fever, Rheumatoid Arthritis, Sarcoidosis, Scleroderma, Sjögren's Syndrome, Stiff-Man Syndrome, Takayasu Arteritis, Temporal Arteritis/Giant Cell Arteritis, Ulcerative Colitis, Uveitis, Vasculitis, Vitiligo, or Wegener's Granulomatosis. In a preferred embodiment, the autoimmune disease is insulin dependent diabetes (also known as autoimmune diabetes).

In particular embodiments, the neurodegenerative disease is a neurological disorder selected from the group consisting of Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Huntington's disease, cerebral stroke, traumatic brain injury, and spinal cord injury. The neurological disease may be the result of an autoimmune condition.

In particular embodiments, the cancer is bladder cancer, pancreatic cancer, cervical cancer, lung cancer, liver cancer, ovarian cancer, colon cancer, stomach cancer, virally induced cancer, neuroblastoma, breast cancer, prostate cancer, renal cancer, leukemia, sarcomas, myeloma, or a carcinoma.

In other embodiments, the method treats cachexia, sarcopenia, reduces chemotherapy-induced inflammation, reduces sepsis, or reduces cancer cell proliferation.

In particular embodiments, the inflammatory condition is osteoarthritis, transplantation related inflammation, cytokine storm, ulcerative colitis, Crohn's disease, proctitis, microscopic colitis, allergic eosinophilic gastroenteritis, food allergies, pill induced esophagitis, celiac disease, recurrent polyps, hemorrhoids, bacterial sepsis, sterile inflammation, an immunological condition, graft versus host disease, or trauma related tissue injury.

Any of the diseases and disorders discussed above in connection with any specific cell type can be treated using an apparatus or device of the invention and any of the other disclosed cell types in the application.

In some embodiments, the method prepares the organ or tissue for transplantation or improves transplantation outcome. In particular, the organ or tissue is *in vivo* or *ex vivo*.

In some embodiments of the method, the organ is or is from lung, heart, liver, bladder, brain, blood vessels, skin, eye structures, gut, bone, muscle, ligament, cartilage, esophagus, pancreas, intestines, gallbladder, bile duct, fallopian tubes, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, ureter, urethra, or uterus.

In other embodiments, the method promotes the proliferation of cells in the organ, tissue, or subject, such as hematopoietic stem cells.

A fifth aspect of the invention features a kit including the apparatus or device of the first aspect of the invention and a cell culture medium or PBS.

In some embodiments, the cell culture medium is in the apparatus or device.

In other embodiments, the cell culture medium is in a container in the kit.

In some embodiments, the kit further comprises a heating unit.

In other embodiments, the immortalized cells are present in the apparatus or device or are present in a container within the kit.

In some embodiments, the kit further comprises a reservoir for collecting effluent medium. In an embodiment the effluent medium can be used for analysis, research, or therapeutic use.

Definitions

The term "subject" means a vertebrate, such as a mammal, such as a human. Mammals include, but are not limited to, humans, dogs, cats, horses, cows, and pigs.

The term "matrix material" includes any material useful as a cell support (e.g., in a device or apparatus of the invention) that allows the flow of PBS or medium around the attached cells.

The term "hollow fiber" is intended to include hollow structures (of any shape) containing pores of defined size, shape and density for use in delivering nutrients (in solution) to cells contained within an apparatus or device of the invention and for allowing bioactive molecules (in solution) produced from cells contained within the apparatus or device to flow out of the apparatus or device and into an organ, tissue, or subject connected to the apparatus or device. For purposes of the invention, hollow fibers may be constructed of a resorbable or nonresorbable material. Fibers include, but are not limited to, tubular structures.

The term "induced pluripotent stem cells (IPS)" means somatic cells that have been reprogrammed, for example, by introducing exogenous genes that confer on the somatic cell a less differentiated phenotype. These cells can then be induced to differentiate into less differentiated progeny. IPS cells have been derived using modifications of an approach originally discovered in 2006 (Takahashi et al., *Cell* 131:861-872, 2007; Yamanaka, S. et al., *Cell Stem Cell,* 1:39-49, 2007). For example, in one instance, to create IPS cells, scientists started with skin cells that were then modified by a standard laboratory technique using retroviruses to insert genes into the cellular DNA. In one instance, the inserted genes were Oct4, Sox2, Klf4, and c-myc, known to act together as natural regulators to keep cells in an embryonic stem cell-like state. These cells have been described in the literature. See, for example, Wernig et al., *PNAS,* 105:5856-5861 (2008); Jaenisch et al., *Cell,* 132:567-582 (2008); Hanna et al., *Cell,* 133:250-264 (2008); and Brambrink et al., *Cell Stem Cell,* 2:151-159 (2008). In another example, iPS cells can be created after mRNA for the reprogramming factors are introduced into cells (Warren et al., *Cell Stem Cell* 7:618-630, 2010).

These references are incorporated by reference for teaching IPSCs and methods for producing them. It is also possible that such cells can be created by specific culture conditions (exposure to specific agents).

DESCRIPTION OF THE DRAWINGS

FIG. 1 describes several cell therapy considerations regarding traditional cell therapy using exogenous cell infusion (left) and cell therapy of the invention using an *ex vivo* cell therapy apparatus or device of the invention (right). Cell therapy considerations 2 to 10 associated with traditional cell therapy may be collapsed into a single consideration when the cell therapy apparatus or device is used, which saves time and decreases the complexity of cell therapy.

FIG. 2A is an image showing a prototype cell therapy apparatus or device (e.g., which can be used *ex vivo*) that can hold 50-150 million MSCs. An inlet port is shown at the upper left and an outlet port is shown at the lower right. The white material is a matrix for cell attachment. The direction of fluid flow through the apparatus or device is depicted by the arrows. The outlet port also includes two tandem filters to prevent any cells from the device from transferring to the tissue, organ, or recipient.

FIG. 2B is an image of another prototype cell therapy apparatus or device (e.g., which can be used *ex vivo*) constructed of sheets of fluorinated ethylene propylene copolymer (FEP). In this image, the inlet port is at the bottom and fluid flows from a perforated tube. The matrix is in the middle and is composed of poly vinyl acetal foam. The outflow is from the top.

FIGs. 3A and 3B are images showing that matrix attachment improves the viability of MSCs *in vivo*. FIG. 3A shows allogeneic MSCs infused by catheter in a dog infarct model and imaged *in vivo*. Only a small portion of the MSCs homed to the infarct and the detectable number of MSCs decreased by 99% in one week (Kraitchman et al., *Arterioscler. Thromb. Vasc. Biol.* 29:1025-1030, 2009). FIG. 3B shows that allogeneic MSCs persist at 16 weeks when implanted on a matrix. Shown are 100s of CM-Di-I labeled allogeneic MSCs on HA/TCP matrix.

FIG. 4 is an image showing that exosomes from hMSCs which contain factors travel long distances in the body. Exosomes are 30-200 μm in diameter, bar=100 μm.

FIG. 5 is an illustration showing the factors from hMSCs that modulate immune responses and bacterial sepsis.

FIG. 6 is a photograph showing the size of a therapeutic dose of 100 million hMSCs.

FIG. 7 is a schematic showing an *ex vivo* cell therapy system within the scope of the invention. The schematic shows a reservoir, (R), containing PBS or nutrient media which flows into the apparatus or device from the top. Cell stimulating molecules and/or drugs can be added to the fluid contained in the reservoir. The apparatus or device provides (A): a porous matrix seeded with immortalized cells, which continuously produce important molecules into the media or can be stimulated to make additional molecules. The apparatus or device retains the immortalized cells as resident cells and maintains their viability. The apparatus or device also provides (B): a triple filter in the outlet port containing a 1 μm pre-filter, a 0.48 μm intermediate filter, and a 0.22 μm final filter. The triple filter allows the therapeutic

molecules produced by the immortalized cells in the apparatus or device to be transferred to the subject. The transferring process can be done using, e.g., a fluid pump (C). The schematic also shows that the *ex vivo* cell therapy system can be attached in many ways to a subject (D), such as to the subject's circulation by way of a central vein or a peripheral vein or to the subject's organ or tissue by way of a catheter.

DETAILED DESCRIPTION OF THE INVENTION

The invention features an apparatus or device for cell therapy. The apparatus or device contains immortalized cells (e.g., immortalized stem or progenitor cells, such as human stem or progenitor cells, in particular, human MSCs) on a matrix that can be used to deliver bioactive factors produced by the immortalized cells into a subject, organ, or tissue when the apparatus or device is fluidly connected to the subject, organ, or tissue (e.g., via an inlet port and an outlet port, which can be connected to a major or minor artery or vein, or to other vessels, or to a collecting sinus of the organ or tissue). The apparatus or device may be implanted or present outside the body of a subject (extracorporeal) and attached to an *in vivo* organ or tissue via tubing or catheters. The attachment may occur proximal or distal to the *in vivo* organ or tissue of the subject that needs treatment. The apparatus or device may also be connected to the subject such that fluid from the apparatus or device flows into the subject's systemic circulation. The apparatus or device may also be attached directly to an *ex vivo* organ or tissue (e.g., an organ or tissue that is removed from a donor, e.g., for transplantation into a recipient). When used in transplantation procedures, the apparatus or device may be used to improve transplant outcomes. This apparatus or device may also be used to treat other medical conditions, including, e.g., inflammatory conditions, autoimmune disease, respiratory conditions, cardiac conditions, chronic heart disease, acute respiratory distress, trauma, organ failure, sepsis, neurodegenerative conditions, and cancer.

Fluid may be circulated through the apparatus or device and into the organ, tissue, or subject. The organ or tissue may be separated from the systemic circulation of the subject, but remain in the patient (e.g., the organ or tissue is not removed from the patient). Alternatively, the apparatus or device may be connected to an organ or tissue that has been removed from a subject (e.g., a donor). The apparatus or device may also be connected to an organ, tissue, or subject for a limited period of time (e.g., during a procedure that lasts 5 minutes to up to 2-5 hours or more (e.g., 24-72 hours or more)). The apparatus or device may also be connected to an organ, tissue, or subject and fluid re-circulated as needed. If desired, the device may be turned on and off such that fluid circulation can be controlled without removing the device from the subject. Alternatively, the device may be detached and reattached, as needed. The device can be replaced with a fresh device between treatments, as needed.

Cellular therapy considerations

For the practicing physician, although they may recognize the therapeutic value of cell therapy, it is not a simple decision to use new approaches, including cell-based therapy, to treat a patient with a particular condition. Most physicians do not receive appropriate training for cell transplantation, such as for bone marrow or mobilized hematopoietic progenitor cell transplantation, although they are familiar with the terms. There are complexities to cellular therapies which most physicians do not have time to consider and explain to the patient and this is an impediment to using cellular therapy. Many of these

considerations are summarized in FIG. 1(left column). Each of the 12 cellular therapy considerations is described below. These cellular therapy considerations represent a complex set of problems that physicians must address. The cell therapy apparatus or device of the invention, which contains resident immortalized cells, solves many of these issues, as is described below. The cell therapy apparatus or device containing resident immortalized cells allows the non-expert to apply powerful cellular therapy that can reduce morbidity and mortality.

1. Clinical Indication - The physician must first consider the patients general health, medical history, age, and immediate clinical indications.

2. Identity/Cell Type - The physician must identify the appropriate cell therapy and be familiar with the various cell choices, their availability, and characteristics of the cells that make them useful as a therapeutic. Different cells can produce different quantities of cytokines and factors, and cells produced in different batches can have different levels of production. The age of the cells is important, as this can affect their production of bioactive cytokines and factors. As there are many cells that may have therapeutic potential, choosing an appropriate cell type can be a complex consideration. The cell therapy apparatus or device containing immortalized cells solves this problem by providing a consistent cell type that remains similar from batch to batch. For different therapeutic indications, it may be optimal to use a particular type of immortalized cell in the apparatus or device, as is discussed below.

3. Delivery - The mode of delivery to the patient must be determined. The cells may be delivered alone, in a special medium, on microspheres, or implanted on a solid matrix. The cells may be needle injected locally into damaged tissue or delivered systemically. The cell therapy apparatus or device containing resident immortalized cells solves these problems since the cells are contained and only the cells' products are infused to the recipient via an intravenous (i.v.) connection for systemic delivery or via a catheter that directs the cell products to the tissue or organ of interest to improve repair, function, and regeneration.

4. Dosage - The physician must consider the safe and effective therapeutic dose to give a patient since there is no way to remove the cells once delivered. Also, as many of the cells do not survive very long after delivery, the delivered dose may not be what the doctor intended. With injected cells, there is no way to test the effective dose in each recipient and there is no assurance that the same dose will stay the same with a new batch of cells the next time or in a new recipient. The cell therapy apparatus or device containing immortalized cells solves this problem by providing a consistent dose of cells in a favorable environment so the cells continue to produce cytokines and factors consistently.

5. Toxicity – Toxicity is always a consideration with effective, powerful medicines including cellular therapy. The effective dosage is usually balanced against its toxicity. Since a good portion of the delivered cells die when delivered by current therapies, it is difficult to know whether cell death increases or decreases the associated toxicity. Similarly, the level of toxicity may be changed if more cells survive delivery. Once delivered, there is no way to reduce the dose or the potential toxic effects resulting from

apoptosis of the delivered cells. The cell therapy apparatus or device containing resident immortalized cells solves this problem by keeping the cells in a favorable environment so their production of cytokines and growth factors is consistent and predictable. If there are signs of toxicity in the recipient, the apparatus or device can be disconnected or removed and, if necessary, replaced with a "fresh" apparatus or device.

6. Biodistribution – The location, action, and survival of the delivered cells are important factors to consider in cell therapy. Once delivered, the cells cannot be removed or be moved to another location inside the body. The cell therapy apparatus or device containing immortalized cells solves the biodistribution problem since cells reside in a single known location (i.e., the apparatus or device). The cells in the apparatus or device can be tested periodically to assess their viability. It is also possible to "relocate" the apparatus or device and cells to another site of interest by making a new vascular connection in the desired location.

7. Degradation - The vast majority of delivered cells do not find a site of engraftment and will die (apoptose) and be removed over time by macrophages. Therefore, the dose of cells undergoes continuous degradation. Even those limited cells that are able to find a site of engraftment may be recognized as foreign and rejected by the immune system. The cell therapy apparatus or device containing immortalized cells solves this problem by keeping the cells in a favorable environment inside the apparatus or device, where their viability can be ascertained and their production of bioactive factors can be analyzed over time.

8. Clearance - Like many of the aforementioned considerations, it is difficult to know where and when the delivered cells are cleared. The cell therapy apparatus or device containing resident immortalized cells solves this problem. When the apparatus or device is disconnected or removed from the recipient, the cellular therapy ends immediately.

9. Long Lead Time - One of the critical considerations in deciding whether to use cellular therapy is how fast the therapy can be initiated. Certain therapies require finding an appropriate donor, obtaining consent, harvesting the tissue or cells from the donor, isolating the cells, growing them in culture, testing their sterility, pyrogenicity, and other safety parameters, all before cell therapy can be initiated. The cell therapy apparatus or device containing resident immortalized cells solves this problem by being available "off-the-shelf" and ready to go with very little lead time.

10. Reproducibility – A medicine should offer reproducible therapeutic benefits, but current cell therapy cannot be applied reproducibly because of cell and donor variability. A cellular therapy product is a complex product and its characteristics can be difficult to reproduce. The cell therapy apparatus or device containing resident immortalized cells solves this problem because the immortalized cells can be standardized so that they are substantially the same time and time again in devices used for the same therapy. Furthermore, the cells can be stored frozen, and last almost indefinitely, providing substantially the same standardized cells for use in the apparatus or device over time.

11. Clinical Outcome - As with all therapies, clinical outcome is the most important consideration and is dependent on many of the factors discussed above that are outside of the physician's control. The cell therapy apparatus or device containing resident immortalized cells eases many of the considerations mentioned above and therefore would be able to provide more predictable clinical outcomes.

12. Repeat Therapy - If there is indication that the therapy might be working the decision whether to re-treat the patient is a significant consideration. Normally, given the complexity of cellular therapy, the decision is difficult because all the considerations described above must be considered once again. The cell therapy apparatus or device containing resident immortalized cells makes this decision easier because the apparatus or device reduces many of these risks and considerations, as outlined above.

With these factors in mind, the features of the cell therapy apparatus or device containing resident immortalized cells will be discussed.

Apparatus for cellular therapy

The invention features an *ex vivo*/extracorporeal or implanted cellular therapy apparatus or device that avoids infusion of exogenous cells, but allows their products, e.g., secreted cytokines, chemokines, and growth factors, to be transferred to the recipient or recipient's cells, tissues, or organs. Rather than infusing the cells directly, the cellular therapy apparatus or device retains the cells within the apparatus or device (as resident cells attached to a substrate, e.g., a matrix) and maintains their viability therein. The cell therapy apparatus or device by itself allows easy handling of the cellular therapy material, and prevents the recipient from receiving the cells directly, eliminating many of the perceived risks of cellular therapy discussed above. The cell therapy apparatus or device containing resident immortalized cells (described in detail below) substantially eliminates variability in many of the cell parameters that may vary from batch to batch, such that the cellular characteristics of the apparatus or device will be substantially the same each time, which facilitates quality control and reproducibility.

The apparatus or device may be connected to the recipient patient via a central or peripheral vein or artery, to a collecting sinus, or via a cut down procedure to access the cells, tissues, or organs of interest. Accordingly, the apparatus or device provides direct infusion of bioactive factors that are secreted by the resident cells of the apparatus or device.

As for therapeutic or industrial application, the cellular therapy apparatus or device can be removed, discontinued, or detached from the recipient or patient at any time, which is not possible when the cells are directly infused to the recipient or patient. To ensure a reproducible production of bioactive factors from device to device, immortalized hMSCs, stem or progenitor cells, or other cells may be used as the resident cells in the apparatus or device. Having the cell therapy apparatus or device should provide greater control and deeper understanding of the activities of the cells (e.g., hMSCs) used during treatment and allow industrial optimization. Two prototypes of this *ex-vivo*/extracorporeal cellular therapy apparatus or device are available (described in detailed below). The prototypes are provided to exemplify the apparatus or device of the invention, but should not be considered as limiting the design or scope of the apparatus or device of the invention in any way. Because the resident cells in the apparatus or device are not freely

infused to the recipient, the cell therapy apparatus or device is inherently safer than most current methods for providing cellular therapy.

Other devices that could be adapted or modified as necessary to include resident immortalized cells (e.g., hMSCs) for use in this invention are described in U.S. Patent Nos. 6582955, 8048419, and 8313944, U.S. Patent Application Publication Nos. 2012/0276518, 2007/0269489, and 2009/0081296, and International Publication Nos. WO 2013/071015, WO 2012/110178, and WO 2009/047300, each of which is incorporated herein by reference in its entireties.

Prototype of cell therapy apparatus or device

FIGs. 2A and 2B each shows a non-limiting prototype of a cell therapy apparatus or device of the invention. For ease of handling, the size and shape of the apparatus or device is similar to a cell phone (e.g., about 3 inches by 5 inches by 0.75 inches). The Plexiglas® prototype apparatus or device of FIG. 2A is constructed by machining a cavity to hold a suitable matrix material (white in FIG. 2A) to support resident cell attachment and viability. The matrix material is encased in a clear container that protects the internal constituents. The apparatus or device also contains an inlet, outlet, and Z-shaped fluid flow path to allow fluid to flow through the matrix material. In a non-limiting example of the apparatus or device of the invention, the outlet of the apparatus or device has a 1 µm pre-filter, a 0.48 µm intermediate filter, and a 0.22 µm final filter. A second prototype constructed as a "flexible bag" is shown in FIG. 2B and is made from sheets of fluorinated ethylene propylene co-polymer (FEP) fused at the edges and containing a polyvinyl acetal foam matrix upon which the cells reside. The culture medium used in the apparatus or device is pre-gassed with 5% CO_2 and exchanged every few days, or can be continuously perfused by a pump. A constant flow rate of 0 to 1.5 ml/min is easily achieved with a peristaltic or constant flow pump. A programmable fluid flow/fluid warmer is available and can be used to maintain the apparatus or device at a constant temperature (e.g., in the range of, e.g., 20-37°C (e.g., 30-37°C). A schematic illustrating an example of an *ex vivo* cell therapy system within the scope of the invention is shown in FIG. 7.

The apparatus or device can be made in different sizes and contain 50,000-200 million, such as 50-200 million, immortalized cells. A typical therapeutic dose for heart ischemia or lung injury is about 100 million hMSCs. The photograph in FIG. 6 shows the size of a therapeutic dose of 100 million hMSCs. The cell therapy apparatus or device could be available off-the-shelf to provide a safe treatment, which could be started, stopped, and disconnected when treatment is completed, which is not the case for injected cells. Moreover, the extended viability of the cells in the cell therapy apparatus or device compared to injection/systemic infusion of exogenous cells suggests greater therapeutic potential from a "dose." Images in FIGs. 3A and 3B show that matrix attachment improves the viability of MSCs *in vivo*. Given the large number of cellular therapy clinical trials underway, the cell therapy apparatus or device containing immortalized cells may be an important addition to the arena of cellular therapy.

The apparatus or device retains the exogenous immortalized cells (e.g., hMSCs) as resident cells and transfers the therapeutic substances, e.g., bioactive factors, produced by the immortalized cells such as cytokines, chemokines, and anti-inflammatory agents to the cells, tissues, and organs of the recipient. The resident cells can maintain viability on many different kinds of suitable matrix materials. Hollow fiber technology has been available for many years and hMSCs have been grown in such bioreactors. Hollow

fiber bioreactors and cells that are grown in such reactors are described in U.S. Patent Publication No. US 20120308531, which is incorporated by reference in its entirety.

The matrix material or hollow fibers for use in the apparatus or device of the invention can be pre-coated with one or more extracellular matrix proteins, for example, MATRIGEL®, laminin, fibronectin, or collagen, to enhance cell attachment. Extracellular matrix proteins may be attached to a cell support (e.g., a matrix material or to the internal and/or external surface of the fibers). Generally, extracellular matrix protein may be attached to the surface by any of the methodologies as described in U.S. Pat. No. 5,872,094 and U.S. Pat. No. 6,471,689, both of which are incorporated herein by reference for teaching these methodologies. The matrix material or hollow fibers should be suitable for the delivery of nutrients and removal of biological agents from the apparatus or device to the organ, tissue, or subject to which the apparatus or device is connected.

The matrix material or hollow fibers may be any shape, for example, they may be round and tubular or in the form of concentric rings. The matrix material or hollow fibers may be made up of a resorbable or non-resorbable membrane. For example, suitable components of the matrix material or hollow fibers include polydioxanone, polylactide, polyglactin, polyglycolic acid, polylactic acid, PLGA, polyglycolic acid/trimethylene carbonate, cellulose, methylcellulose, cellulosic polymers, cellulose ester, regenerated cellulose, pluronic, collagen, elastin, and mixtures thereof. The large variety of suitable materials is well-known in the art and is represented by a large body of literature. Examples of these materials have been described in U.S. Pat. Nos. 4,220,725; 4,184,922; 4,200,689; 3,821,087; 3,883,393; 4,184,922; 4,200,689; 3,997,396; 4,220,725; 4,999,298; 4,804,628; 5,126,238; 5,656,421; 5,162,225; 5,622,857; 5,627,070; 6,001,585; 6,911,201; 6,933,144; 7,534,609; and U.S. Publications 2007/0298497; 2008/0220523; 2001/0044413; 2009/0196901; 2010/0233130; 2009/0191631; 2005/0032218; 2005/0003530; 200310224510; 2006/0205071; 2010/0267134; 2008/0206733; 2010/0209403, 2008/0213894; 2008/0220522; 2008/0227190; 2008/0248572; 2008/0254533; 2010/0144037; and 2010/0042260 all of which are incorporated by reference for teaching these materials. The criteria for matrix materials includes, but is not limited to, the following: not toxic to the cells; porous for the removal of biological agents and materials and the reception of nutrients and, in instances where collection of components secreted by the cell is desired, porosity is adjusted for that parameter; relatively insensitive to temperature changes, i.e., thermally stable; able to retain shape integrity.

MSCs have also been grown on multiple types of matrix materials and scaffolds, even titanium, *in vitro* and *in vivo*. Multiple apparatus or device designs may be used for the apparatus or device of the invention (e.g., hollow fiber, non-woven, woven, beads), and many different fluid flow paths (e.g., straight, U-shaped, Z-shaped, and multichannel) may be used. The apparatus or device may be relatively small in size, of simple construction, made from non-proprietary materials, and designed to hold about 50,000-200 million, such as 50-200 million, such as 50-150 million, immortalized cells (e.g., hMSCs).

The matrix holding the immortalized cells (e.g., hMSCs) can be made of several materials, but we have used a nonwoven polylactic acid (PLA), a polyvinyl acetal foam, and a collagen sponge with similar success. In one embodiment, the cell therapy apparatus or device may be formed of polycarbonate. After the chosen matrix is inserted, the apparatus or device is sealed, and gas sterilized (although other known sterilization techniques may be used). In another embodiment, the fluorinated ethylene propylene

copolymer (FEP) sealed bag containing the polyvinyl acetal foam (see, e.g., FIG. 2B) is steam sterilized and dried before being loaded with cells.

The immortalized cells (e.g., im-hMSCs) can be inoculated into the apparatus or device in a biological safety cabinet and the cells may be grown for 7-14 days to achieve a desired number of captured immortalized cells (e.g., im-hMSCs). In some examples, the immortalized cells (e.g., im-hMSCs) can also be grown outside the apparatus or device and then inserted into the apparatus or device once the desired number of cells are ready. It is important to use immortalized cells in the apparatus or device because in order to make a reproducible cell therapy apparatus or device that responds in a consistent fashion, the cells within the apparatus or device should be substantially the same from batch to batch. Newly isolated stem cells can be used each time, but the apparatus or device parameters will likely not be as consistent.

The cell therapy apparatus or device connected to the blood supply may need to be just proximal to the organ of interest to offer greatest therapeutic potential. This may require surgical intervention, such as a procedural cut-down to access the needed blood vessel. Surgeons have assured us that this is not difficult and has small but real risks commonly associated with vascular surgery. For disseminated diseases, such as GvHD, a cell therapy apparatus or device containing immortalized cells (e.g., im-hMSCs) may reduce inflammation, such that a drug or a small molecule may provide therapeutic control or, e.g., a reduced dose of infused MSCs can then offer sufficient control, thereby reducing risk(s) to the recipient.

Alternatively, other designs of the apparatus or device may be available to use in addition to the prototype cell therapy apparatus or device described in detail above and in FIGs. 2A and 2B. In some embodiments, the apparatus or device may contain multiple inlet and outlet openings or ports. One or more of the ports may be used to collect effluent medium or add, e.g., materials, fluids, bioactive factors, agents, and cell culture medium to the apparatus or device. In other embodiments, the apparatus or device may also be in the form of a culture bag (e.g., a gas-permeable culture bag).

The cell therapy apparatus or device as described herein is an early evolution of cellular therapy that does not use direct infusion of therapeutic cells. We have envisioned several versions of the apparatus or device, but the current version should not be limited to any particular application. The described apparatus or device can be used in therapeutic or industrial methods, as well as to improve our current understanding of stem or progenitor cell therapy (e.g., hMSC therapy). The apparatus or device here, which may be about the size of a cell phone, may contain up to 200 million (e.g., 5-200 million) immortalized stem or progenitor cells (e.g., hMSCs) that can produce therapeutic effects by transferring their bioactive factors, e.g., cytokines, chemokines, and anti-inflammatory agents, when connected to the patient's circulation or to an organ or tissue. The apparatus or device can be disconnected when treatment is over or restarted as needed without subjecting the patient (or an organ or tissue) to injected cells and their unknown long term effects.

Immortalized cells of the invention

The ability to form clonogenic colonies is a characteristic of somatic stem cells including hematopoietic stem cells and MSCs. Approaches to translate the therapeutic potential of these cells to clinical applications suffer from the lack of supply of suitable cells, their short life-span, and high inter-

donor viability within stem/progenitor cell preparations. For example, the low amount of hMSCs in bone marrow extracts (0.001 to 0.1 % of nucleated cells) typically necessitates an *in vitro* cell growth phase prior to use. This *in vitro* cell growth phase is limited by the replicative senescence phenomenon occurring under *in vitro* culture conditions, after 25 to 60 population doublings (PD). Also, the differentiation potential of hMSCs displays significant variations from donor to donor and this may reflect other undesirable variations from preparation to preparation. Overall, the low supply of hMSCs combined with their short life-span and the high inter-donor variability in characteristics limits the practical therapeutic potential of these stem cells.

To overcome the difficulties of using these cells clinically, immortalization of the cells so they may grow for many more population doublings or indefinitely, is a technique that can offer an unlimited supply of well-characterized multipotent cells with many uses. Immortalized stem/progenitor cells can be propagated in large numbers with the pluripotency intact, although pluripotency and differentiation potential may not be required for most of the envisioned applications. Immortalized cells (e.g., hMSCs) can be produced using methods known in the art. In general, gene expression levels of immortalized cells (e.g., im-hMSCs) remain stable almost indefinitely, which is not true for stem/progenitor cells that are not immortalized.

Immortalized cells or stem cells of the invention are cells that may maintain their multipotencies, produce useful factors, and/or have the capacity to grow indefinitely. Methods of producing immortalized cells include taking non-immortalized cells and introducing an "immortalizing gene" to the genome of the cells. Non-integrating vectors that do not introduce immortalizing genes into chromosomes, such as episomal plasmids and mini-chromosomes, are also known in the field. Methods of producing immortalized cells are well known in the art and are described in detail below or in publications incorporated herein by reference.

Human mesenchymal stem cells (hMSCs) derived from the bone marrow may be used as the resident stem cells in a cell therapy apparatus or device of the invention. These stem cells are defined as flow cytometry positive for CD73, CD90, and CD105, and negative for hematopoietic markers (e.g., CD34) and are able to stably differentiate *in vitro* into osteoblasts, adipocytes, and chondrocytes (Pittenger et al., *Science* 284:143-147, 1999). The multidifferentiation capacity of hMSCs makes them promising candidates for regenerative medicine. The chondrogenic and osteogenic potential of hMSCs is useful for the repair of damaged articular cartilage but also for bone tissue engineering by recapitulating intramembranous or endochondral ossification processes.

While hMSCs can participate in building tissue, particularly bone, they also produce multiple anti-inflammatory agents, growth factors, and cytokines, as is described below. Many factors are produced constitutively in substantial amounts by hMSCs, and interaction of hMSCs with other cell types causes additional factor release, either from the hMSCs or the other cells (Haynesworth et al., *J. Cell. Physiol.* 166:585-92, 1996; Majumdar et al., *J. Cell. Phys.* 176:57-66, 1998; Aggarwal and Pittenger, *Blood* 105:1815-1822, 2005; Klyushnenkova et al., *J. Biomedical Sciences* 12:47-57, 2005; Di Nicola et al., *Blood* 99:3838-43, 2002; Kraitchman et al., *Circulation* 112:1451-61, 2005; Walczak et al., *Stroke* 39:1569-74, 2008). The hMSC-derived anti-inflammatory agents, growth factors, and cytokines have profound effects on the local environment of tissues and organs, but also can have effects systemically, such as in the case of patients suffering from GvHD. Moreover, hMSCs respond to their neighboring cells

and modulate tissue responses gradually and appropriately over time, such as in the case of hMSC modulation of cardiac remodeling when the cells are injected following an infarct (Zhao et al., *Stem Cells Transl. Med.* 1:685-695, 2012). It is well-known in the art that hMSCs are biologically responsive to their local environment *in vivo*. As an example, hMSCs produce vascular endothelial growth factor (VEGF) but upon interaction with peripheral blood mononuclear cells, the amount increases by three fold (Aggarwal and Pittenger, *supra*).

Other names have been used to refer to MSCs or cells that are highly related. MSC-like cells can be isolated from different tissue sources and produced in different labs and by different commercial organizations, although the cells have similar cell properties, uses, immunological effects, and produce similar bioactive factors. The names given to these MSC-like cells include mesenchymal stromal cells, marrow stromal cells, mesenchymal precursor cells (MPC) (Hamamoto et al., *Ann Thorac Surg.* 87:794-801, 2009), marrow stromal stem cells (Gronthos et al., *Journal of Cell Science*. 116:1827-1835. 2003), multipotent adult progenitor cells (MAPC) (Verfaillie et al., *Journal of Clinical Investigation*. 109:1291-1302, 2002; Verfaillie et al., *Journal of Experimental Medicine*. 204:129-139, 2007; Kovacsovics-Bankowski et al., *Cytotherapy* 10:730-742, 2008; Yasuhara et al., *J. Cereb. Blood Flow Metab.* 28:1804-1810, 2008), immunoselected mesenchymal progenitor cells, skeletal stem cells (SkSCs) (Krebsbach et al., *Dent. Educ.* 66:766-773, 2002), stromal stem cells (Gronthos et al., *Journal of Cell Science* 116:1827-1835. 2003), adipose-derived mesenchymal stem cells, adipose derived stromal stem cells (Zannettino et al., *J. Cell. Physiol.* 214:413-421, 2008), multilineage adipose cells (Zuk et al., *Tissue Eng.* 12:2813-2823, 2001), marrow isolated adult multilineage inducible (MIAMI) cells (D'Ippolito et al., *J. Cell. Sci.* 117:2971-2981, 2004), stem cells from human exfoliated deciduous teeth (SHED) (Miura et al., *Proc. Natl. Acad. Sci. U.S.A.* 100:5807-5812, 2003; Yamaza et al., *Stem Cell Res. Ther.* 1:5, 2010), dental pulp stem cells (DPSCs) (Zuk et al., *Tissue Eng.* 12:2813-2823, 2001), periodontal ligament stem cells (PDLSCs), stem cells from apical papilla (SCAP), and dental follicle precursor cells (DFPCs). Additional names for highly related cells include adult progenitor cells (APCs), pluripotent placental derived adherent cells (PDAC®, PDA-001 or PDA-002; Celgene Cellular Therapeutics, Inc.), culture-expanded MSCs from bone marrow (PROCHYMAL® and CHONDROGEN®; Osiris Therapeutics, Inc.), MULTISTEM® (Athersys Inc.), placental expanded (PLX) cells (Pluristem Therapeutics, Inc.), and ALD-201, ALD-301, and ALD-401 (Cytomedix, Inc.). Each of these cells and cell types may be used in the apparatus or device of the invention as resident cells. The cells may be used as is or they may be immortalized prior to use.

The concept that all these cells and the bone marrow MSCs may have a common developmental origin has been widely discussed, along with the strong possibility that similar cells known as mural cells, microvascular pericytes or simply pericytes are present along all blood vessels in the body, and therefore they are available as reparative cells at the first sign of injury (Meirelles et al., *J. Cell Sci.* 119:2204-2213, 2006; Crisan et al., *Cell Stem Cell.* 3:301-313, 2008; Chen et al., *Cytokine Growth Factor Rev.* 20:429-434, 2009). The MSC or MSC-like cells are also reported to stimulate blood vessel formation and the appearance of other important reparative cells such as c-Kit+ stem cells in damaged tissue, most notably the heart (Orlic et al., *Proc. Natl. Acad. Sci. U.S.A.* 98:10344-10349, 2001; Beltrami et al., *Cell* 114:763-776, 2003). Other stem/progenitor cell populations that have been isolated from hearts and characterized are termed the cardiosphere derived cells (CDCs) and the related cardiosphere progenitor cells (CSpc) (Chimenti et al., *Circ. Res.* 106:971-980, 2010). These are thought to have some potential for

differentiation to cardiac cells, but to a large extent their mode of action is the release of paracrine factors such as VEGF, HGF and insulin-like growth factor-1 (IGF-1) (Li et al., *J. Am. Coll. Cardiol.* 59:942-953, 2012). These cells and cell types can also be used in an apparatus or device of the invention as resident cells. The cells may be used as is or they may be immortalized prior to use.

Moreover, other multipotent cells that would prove useful in the cell therapy apparatus or device include Hox 11^+ cells (e.g., $Hox11^+$, $CD45^-$ cells from spleen) as described by Faustman et al. (Faustman et al., *Int. J. Biochem. Cell Biol.* 42:1576-1579, 2010; Lonyai et al., *Horm. Metab. Res.* 40:137-146, 2008; U.S. Patent Publication NOs. 8,021,693, 7,582,313, and 8,017,392; incorporated herein by reference). These cells also produce important factors. The Hox 11+ cells are under intense study for their ability to form pancreatic islet cells useful for treating diabetes. Therefore, the cell therapy apparatus or device containing immortalized Hox 11+ cells can be useful for the study and treatment of diabetes. These $Hox11^+$ cells could also be used in the cell therapy apparatus or device, preferably with the cells immortalized, for use in the treatment of diabetes and other autoimmune disease or to promote repair and regeneration of damaged tissue and organs.

Similarly, any cellular use that involves, as part of its mode of action, the expression of cytokines, growth factors, or other molecules, such as those systems that utilize CD34+ cells or their derivatives, alone or in combination with MSC-like cells, such as ixmyelocel from Aastrom Biosciences Inc., could be combined with the apparatus or device of the invention as is or in an immortalized form.

Other cells that could be used with the apparatus or device include, but are not limited to, cells of the lung, heart, liver, bladder, brain, nervous system tissue, blood vessels, skin, eye structures, gut, bone, muscle, ligament, cartilage, esophagus, pancreas, intestines, gallbladder, bile duct, fallopian tubes, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, ureter, urethra, uterus, or fat. These cells could also be immortalized and used in the cell therapy apparatus or device.

In addition to specialized cells, other stem cells that can also be used in the invention include, but are not limited to, embryonic stem cells, induced pluripotent stem cells (IPS), hematopoietic stem cells, intestinal stem cells, osteoblastic stem cells, mesenchymal stem cells, multipotent adult progenitor cells (MAPCs), unrestricted somatic stem cells (USSCs), neural stem cells, epithelial stem cells, bone stem cells, cardiac myocyte progenitor stem cells, skin stem cells, skeletal stem cells, placental stem cells, placental-derived multipotent stem cells, muscle stem cells, endothelial stem cells, umbilical cord stem cells, human umbilical cord perivascular cells (HUCPVCs), adipose stem cells, and liver stem cells.

In some embodiments, mesenchymal stem cells are selected from lung mesenchymal stem cells, Wharton's Jelly mesenchymal stem cells, bone marrow mesenchymal cells, bone marrow stromal cells, umbilical cord mesenchymal cells, spleen mesenchymal cells (e.g., $Hox11^+$, $CD45^-$ cells), adipose derived mesenchymal cells, and pericytes. In some embodiments, the epithelial stem cells are selected from the group consisting of lung epithelial stem cells, breast epithelial stem cells, vascular epithelial stem cells, and intestinal epithelial stem cells. In some embodiments, the skin stem cells are selected from the group consisting of epidermal stem cells and follicular stem cells (hair follicle stem cells). In other embodiments, the neural cells are selected from neuronal dopaminergic stem cells and motor neuron stem cells.

The cell therapy apparatus or device could be used with any or all of these so named cells (or other cells) to perform similar scientific, therapeutic, or industrial uses. Each of these cell types could be immortalized to provide for an unlimited supply of progenitor cells for use in the cell therapy apparatus or

device. The cell therapy apparatus or device containing immortalized progenitor or stem cells, such as MSCs, or those cells and cell types described above that provide repair and regeneration factors may also be useful to study recovery from cerebral stroke, traumatic brain injury, neural injury, or spinal cord injury (SCI), and to provide new therapeutic or industrial uses (Yasuhara et al., *J. Cereb. Blood Flow Metab.* 28:1804-1810, 2008; Chopp et al., *Lancet Neurol.* 1:92-100, 2002; Li et al., *Glia.* 49:407-417, 2005; Busch et al., *Journal of Neuroscience* 31:944-953, 2011; STEPS *Stroke* 40:510-515, 2009).

Methods of producing immortalized cells

Immortalized cells are cells that have the capacity to divide and proliferate indefinitely either *in vivo* or *in vitro* over a long period of time. Various methods of producing immortalized cells are available in the art. Techniques for immortalizing cells are described in WO 2003014320, US 5716830, US 5648219, WO 2013147082, WO2013186264, WO 2002059285, US 20120237607, WO 2000061617, WO 1997032992, WO 1997023602, US 7776587, and WO2013118786, which are incorporated herein by reference in their entireties.

In brief, methods of making immortalized cells generally include introducing an "immortalizing gene," such as an oncogene, a cell proliferation factor gene, a telomerase gene to the cells using virus-based cell transduction methods. Other transduction methods are also available in the art, such as electroporation, liposome-based gene delivery, or Ca^{2+} phosphate precipitation (see, e.g., Wigler et al., *Cell* 16:777, 1979), but virus-based transduction methods generally give higher transduction efficiency. Normally, the "immortalizing genes" and viruses used for transduction are first packaged and secreted into cell culture medium by other cells that are used particularly for the purpose of generating these viruses. The packaged viruses containing an "immortalizing gene" in the culture medium are collected and added to cells to inoculate them. Several viral mediated transduction methods are available in the art, such as adenovirus-mediated transduction, retrovirus-mediated transduction, adeno-associated virus-mediated transduction, lentivirus-mediated transduction, and herpes virus-mediated transduction. The immortalizing genes introduced may or may not be under the control of an inducible promoter. Once the genes are incorporated into the genome of the cell, they can be transmitted to the daughter cells and therefore can be expressed stably over a long period of time. The oncogene or telomerase gene would be able to provide cells with the ability to proliferate indefinitely. After the cells are successfully transduced with immortalizing genes, they are often cultured in the presence of a combination of growth factors to ensure that the introduced gene is active to enable the production of immortalized cells.

In the invention, immortalized stem cells, e.g., im-hMSCs, can be made by utilizing the SV40 T antigen, a proto-oncogene. In one embodiment, the T antigen is constitutively expressed, and the cells grow robustly. Alternatively, an inducible Tetracycline-on large T antigen vector can be used such that the stem cells (e.g., hMSCs) are cultured and produced with doxycycline (dox) present in the culture medium. In this case, once the cells are on the matrix in the cell therapy apparatus or device, dox may be discontinued, which would limit the further proliferation of the cells. The viability of the cells would be maintained in the apparatus or device. Tetracycline-on promoters and vectors expressing T antigen are well known in the field.

Another method for immortalizing stem cells, e.g., hMSCs, utilizes the ecdysone receptor-based inducible gene expression system for promoting expression of the immortalizing gene (see, e.g., U.S. Patent No. 7,776,587, which is incorporated by reference herein).

Bioactive factors produced by stem cells

It is well-known in the art that in addition to differentiating into cells of specialized tissues or organs, stem cells also produce and secrete useful therapeutic substances in the form of cytokines, chemokines, growth factors, or anti-inflammatory agents that can influence their neighboring cells. In particular, some stem cells are not static producers of these substances but respond to the molecules and the chemical environment nearby to modulate their production of certain useful substances.

The apparatus or device of the invention transfers the therapeutic substances or bioactive factors produced by the resident cells in the apparatus or device to the recipient damaged organs or tissues. Bioactive factors produced by mesenchymal stem cells include interleukins (IL-) 1a, 1b, 6, 7, 8, 10, 11, 14 and 15, macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), stem cell factor (SCF-1), leukemia inhibitory factor (LIF), prostaglandin E-2 (PGE-2), transforming growth factor β (TGF-β), hepatocyte growth factor (HGF), stromal cell derived factor-1 (SDF-1), thrombopoetin (TPO), Flt-3 ligand, exosomes containing factors, pro-factors, mRNAs, and immune modulating factors including TGF- β, IL-1α receptor antagonist (IL-1Rag), indoleamine 2,3-dioxygenase (IDO), inducible nitric oxide synthase (iNOS), galectin 1 (Gal-1), human leukocyte antigen G (HLA-G), TNF-α stimulated gene/protein 6 (TSG-6), and antibacterial factors PGE-2 and peptide LL-37. In the invention, the hMSCs are known to produce up to 20 factors that are useful in repairing tissues and organs. For example, factors produced by hMSCs that inhibit inflammation include TGF-β, HGF, PGE-2, Gal-1, iNOS, IL-6, CD73, IL-1Rag, IL-10, HLA-G, IDO, and TSG-6. Growth factors produced by hMSCs include M-CSF, G-CSF, GM-CSF, LIF, SCF, Flt-3 Ligand, TPO, and SDF-1. As can be seen in FIG. 4, exosomes from hMSCs which contain factors travel long distances in the body. At least 11 factors produced by hMSCs are known to reduce inflammatory responses (see FIG. 5, and Aggarwal and Pittenger *Blood* 105:1815-1822, 2005; Pittenger et al., *Cell Stem Cell*. 5:8-10, 2009). In addition, hMSCs also interact with proinflammatory dendritic cells (DC1) to reduce production of TNFα and IL-12, and with anti-inflammatory dendritic cells (DC2) to increase IL-10 and IL-4. Similarly, hMSCs interact with proinflammatory T cells (T_H1) to reduce expression of interferon γ, and with anti-inflammatory T cells (T_H2) to increase expression of IL-4 and IL-5. Importantly, anti-inflammatory Tregs are also increased by hMSCs. Moreover, similar effects are seen *in vivo* on T cell activity and inflammation, and injection of hMSCs at 1-2 million per kg body weight are effective in treating severe GvHD grade III and normally deadly grade IV GvHD (Le Blanc et al., *Lancet* 363:1439-41, 2004).

Tissues and organs that can benefit from factors produced by immortalized stem or progenitor cells and provided by the cell therapy apparatus or device of the invention

As mentioned previously, immortalized stem or progenitor cells, such as hMSCs, and other useful cells can produce many bioactive factors that are able to provide therapeutic benefits. The apparatus or device of the invention provides a way to supply those factors to tissues or organs in need. The organs

that may benefit from the apparatus or device include, but are not limited to, lung, heart, liver, bladder, brain, blood vessels, skin, eye structures, gut, bone, muscle, ligament, cartilage, esophagus, pancreas, intestines, gallbladder, bile duct, fallopian tubes, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, ureter, urethra, uterus, or fat. The tissues that may benefit from the apparatus or device include, but are not limited to, tissues of the lung, heart, liver, bladder, brain, blood vessels, nervous system, skin, eye structures, gut, bone, muscle, ligament, cartilage, esophagus, pancreas, intestines, gallbladder, bile duct, fallopian tubes, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, ureter, urethra, uterus, and fat.

In particular, the apparatus or device of the invention is especially useful in providing benefits to organs that are undergoing transplant surgeries. For example, the apparatus or device may be attached to the donor or recipient extracorporeally, proximal to the organ of interest in the case of the donor. The apparatus or device may also be attached to an organ that is already harvested from a donor and is being transported to a recipient. The apparatus or device may also be connected to the recipient after transplant surgeries. In all cases, the apparatus or device would be able to transfer therapeutic bioactive factors to the organ or recipient and could be used to improve transplant outcomes.

Preventing cytokine storm

There are several medical situations where the body's response is more dangerous than the disease or injury itself. Such is the case when the body is subject to a cytokine storm brought on by an over-stimulated immune system. As described previously, MSCs or other stem cells used in the apparatus or device containing immortalized cells can be powerful producers of growth factors and cytokines, and of factors that modulate immune responses including PGE-2, TGF- β, IL-1α receptor antagonist (IL-1Rag), indoleamine 2,3-dioxygenase (IDO), inducible nitric oxide synthase (iNOS), galectin 1 (Gal-1), human leukocyte antigen G (HLA-G), TNF-α stimulated gene/protein 6 (TSG-6), and antibacterial factors PGE-2 and peptide LL-37.

In medical practice, bone marrow transplantation using bone marrow material or mobilized peripheral blood stem cells may result in the patient experiencing graft versus host disease (GVHD). In GVHD, the newly grafted stem cells produce immune cells that see the host as foreign tissue and attack. The graft produces dendritic cells, T cells, and B cells that conspire to destroy the "foreign" cells around them, and results in tissue damage that can be life threatening if it becomes severe (grade III or grade IV) GVHD. The inflammation, edema, and fever from tissue damage result in additional responses from the body including the profound increase in the release of cytokines that stimulate many responses. This cytokine storm is difficult for clinicians and nurses to manage as so many biochemical and tissue reactions occur simultaneously. It becomes a waiting game to see which reaction becomes life threatening and must be treated most immediately. The patient may "crash' several times before cytokine storm subsides after hours to days. The apparatus or device containing immortalized cells can be used to modulate the immune response that occurs in GVHD and subsequent cytokine storm(s).

Recently, cancer researchers have learned to harness the immune response to attack cancer cells in the body. This is done by isolating a patient's dendritic cells and exposing them to known cancer antigens, which they engulf, process, and present to T cells and B cells to stimulate the immune response against the cancer antigen. When this works properly it can result in the rapid elimination of cancer cells

that express the known cancer antigen from the body. Such patient treatment can be highly successful. However, the accelerated immune response also can result in life threatening cytokine storm that causes rapid and painful physiological changes that are life threatening. All the biochemical and tissue reactions occur rapidly and are difficult for the clinicians/nurses to effectively treat with current therapies. A patient's condition may quickly go from stable to life threatening within tens of minutes. The apparatus or device containing immortalized cells such as immortalized MSCs or other stem/progenitor cells which releases factors that modulate the immune response can be effective in preventing or alleviating life threatening cytokine storm. By utilizing the apparatus or device containing immortalized cells, the physician has another tool to intervene in a situation where there are few choices.

Medical conditions that can be treated with factors produced by immortalized stem or progenitor cells and provided by the cell therapy apparatus or device of the invention

The apparatus or device of the invention can also provide a method of treatment for various medical conditions which include, but are not limited to, an acute disease, a chronic disease, an autoimmune disease, a skin disease, a neurodegenerative disease, a musculoskeletal disease, cancer, a vascular or circulatory disease, an inflammatory condition, a rheumatology disease, cardiac infarction, acute or chronic heart disease, acute or chronic respiratory disease or distress, trauma, sepsis, and organ failure.

In some embodiments, autoimmune diseases that can be treated by using an apparatus or device of the invention include, e.g., Alopecia Areata, Ankylosing Spondylitis, Antiphospholipid Syndrome, Addison's Disease, Hemolytic Anemia, Hepatitis, Behcets Disease, Bullous Pemphigoid, Cardiomyopathy, Celiac Sprue-Dermatitis, Chronic Fatigue Immune Dysfunction Syndrome (CFIDS), Chronic Inflammatory Demyelinating Polyneuropathy, Churg-Strauss Syndrome, Cicatricial Pemphigoid, Limited Scleroderma (CREST Syndrome), Cold Agglutinin Disease, Crohn's Disease, Discoid Lupus, Essential Mixed Cryoglobulinemia, Fibromyalgia-Fibromyositis, Graves' Disease, Guillain-Barré Syndrome, Hashimoto's Thyroiditis, Hypothyroidism, Idiopathic Pulmonary Fibrosis, Idiopathic Thrombocytopenia Purpura (ITP), IgA Nephropathy, Insulin dependent Diabetes, Juvenile Arthritis, Lichen Planus, Lupus, Ménière's Disease, Mixed Connective Tissue Disease, Multiple Sclerosis, Myasthenia Gravis, Pemphigus Vulgaris, Pernicious Anemia, Polyarteritis Nodosa, Polychondritis, Polyglandular Syndromes, Polymyalgia Rheumatica, Polymyositis and Dermatomyositis, Primary Agammaglobulinemia, Primary Biliary Cirrhosis, Psoriasis, Raynaud's Phenomenon, Reiter's Synùrome, Rheumatic Fever, Rheumatoid Arthritis, Sarcoidosis, Scleroderma, Sjögren's Syndrome, Stiff-Man Syndrome, Takayasu Arteritis, Temporal Arteritis/Giant Cell Arteritis, Ulcerative Colitis, Uveitis, Vasculitis, Vitiligo, and Wegener's Granulomatosis. In particular, the autoimmune disease to be treated is type I diabetes.

For example, a clinical trial for autoimmunity tests MSC infusion for the treatment of multiple sclerosis or neuromyelitis optica (inflammation of the optic nerve). These progressive diseases are currently not well controlled. The infusion of MSCs that may provide anti-inflammatory molecules is being tested. Additionally, hMSCs are known to make at least 11 factors that modulate inflammation including TGF-β, PGE-2, HGF, Gal-1, IL-6, IL-10, IL-1Rag, HLA-G, IDO and TSG-6. Each factor can reduce inflammation and the combination of factors can be even more potent, making hMSCs highly hopeful in treating severe or refractory inflammatory and/or autoimmune disorders. The use of the cell therapy apparatus or device containing cells that produce these factors will provide a safer method of treatment

since it does not require injection of the cells into the patient. Moreover, the cells will remain viable in the apparatus or device for a longer period of time than when delivered into the body.

In some embodiments, neurodegenerative diseases that may benefit from the invention include, but are not limited to, Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Huntington's disease, cerebral stroke, traumatic brain injury, and spinal cord injury.

In particular, there is evidence that stem cells from the bone marrow when transplanted into the brain may be effective in slowing the progression of Parkinson's disease. Mesenchymal stem cells produce TGF-β and monocyte chemoattractant protein 1 (MCP-1) and other factors supporting central nervous system cells. Treatment with MSCs can also increase the level of nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) in the brain. In a study of Parkinson's patients with Progressive Supranuclear Palsy, mesenchymal stem cells are harvested from the bone marrow, cultivated so they multiply, and then infused into the arteries that supply blood to the brains of patients. Arterial delivery of cells, which are larger than most capillaries, can result in ischemia and stroke risks. Thus, the use of bioactive factors as provided by the cell therapy apparatus or device containing MSCs or other stem/progenitor cells and not cell infusion can limit such risks.

Furthermore, mesenchymal stem cells are being tested in patients with ALS at several doses given intrathecally (spinal cord delivery). Recipients will receive injection of 10 million cells, 50 million cells, 100 million cells or 2 doses of 50 million or 100 million cells 4 weeks apart. Owing to their production of anti-inflammatory molecules and growth factors, it is envisioned that the cells will improve the clinical outcome. The protocol requires 8 weeks for the isolation and proliferation of the MSCs from the patient before treatment can begin. The use of the cell therapy apparatus or device with its immortalized cells can begin immediately as no waiting period for cell growth is needed.

In one embodiment, the PROCHYMAL® cell product (Osiris Therapeutics, Inc.) could be incorporated in the apparatus or device of the invention. This apparatus or device could be used in the treatment of GvHD, Crohn's Disease, chronic obstructive pulmonary disease (COPD), acute myocardial infarction, diabetes, and acute radiation syndrome (ARS) in a subject (or organ or tissue) in need. Another product, CHONDROGEN® (Osiris Therapeutics, Inc.), could be incorporated in the apparatus or device of the invention. This apparatus or device could be used in the treatment of osteoarthritis.

In another embodiment, the MULTISTEM® cell product (Athersys, Inc.) could be incorporated in the apparatus or device of the invention. This apparatus or device could be used in the treatment of 1) inflammatory and immune diseases, such as diabetes, allergies, rheumatoid arthritis (RA), immune deficiency disorders, inflammatory bowel disease (e.g., IBD, Crohn's, ulcerative colitis), lupus, graft-versus-host disease (GvHD), dermatological conditions (e.g., psoriasis, eczema), pelvic inflammatory disease, pulmonary conditions, scleroderma, transplant rejection, vasculitis, and a range of others; 2) neurological diseases and disorders, such as acute trauma or ischemic injury conditions, such as stroke (e.g., ischemic stroke), traumatic brain injury (TBI), spinal cord injury, and neonatal hypoxic ischemia, as well as episodic, chronic, or progressive neurological conditions, such as depression, schizophrenia, epilepsy, Parkinson's Disease, Multiple Sclerosis, Alzheimer's Disease, migraine, ADHD, cerebral palsy, and other conditions; 3) cardiovascular diseases, such as acute myocardial infarction, atherosclerosis, high blood pressure, peripheral vascular disease, and congestive heart failure (CHF); and 4) osteoarthritis.

In another embodiment, PDA-002 (Celgene, Inc.) could be incorporated into an apparatus or device of the invention. This apparatus or device could be used in the treatment of, e.g., peripheral artery disease and diabetic foot ulcers in diabetic patients, or for other recognized uses to which the PDA-002 cell therapy product has been applied.

In another embodiment, PLX-PAD (Pluristem, Inc) could be incorporated into an apparatus or device of the invention. This apparatus or device could be used in the treatment of, e.g., intermittent claudication (Charcot's Syndrome), which is related to peripheral artery disease, or for other recognized uses to which the PLX-PAD cell therapy product has been applied.

In another embodiment, mesenchymal progenitor cells (MPC) (Mesoblast Ltd.) could be incorporated into an apparatus or device of the invention. This apparatus or device could be used in the treatment of, e.g., myocardial infarction, or for other recognized uses to which the MPC cell therapy product has been applied.

In another embodiment, ALD-401, ALD-301, ALD-201, ALD-151, and ALD-601 cells (Cytomedix, Inc.) could be incorporated into an apparatus or device of the invention. An apparatus or device of the invention containing ALD-401 cells could be used in the treatment of, e.g., stroke, or for other recognized uses to which the ALD-401 cell therapy product has been applied. An apparatus or device of the invention containing ALD-301 cells could be used in the treatment of, e.g., critical limb ischemia, or for other recognized uses to which the ALD-301 cell therapy product has been applied. An apparatus or device of the invention containing ALD-201 cells could be used in the treatment of, e.g., ischemic heart failure, or for other recognized uses to which the ALD-201 cell therapy product has been applied. An apparatus or device of the invention containing ALD-151 cells could be used, e.g., to improve engraftment following cord blood transplants in the treatment of leukemia, or for other recognized uses to which the ALD-151 cell therapy product has been applied. An apparatus or device of the invention containing ALD-601 cells could be used in the treatment of, e.g., inherited metabolic diseases, or for other recognized uses to which the ALD-601 cell therapy product has been applied.

In other embodiments, any of the diseases and disorders discussed above in connection with any specific cell type could be treated using an apparatus or device of the invention that contains any of the other disclosed cell types in the application. For example, an apparatus or device of the invention that contains one or more of the cell types disclosed herein could be used in the treatment of one or more of the following: 1) inflammatory and immune diseases, such as diabetes, allergies, rheumatoid arthritis (RA), immune deficiency disorders, inflammatory bowel disease (e.g., IBD, Crohn's, ulcerative colitis), lupus, graft-versus-host disease (GvHD), dermatological conditions (e.g., psoriasis, eczema), pelvic inflammatory disease, pulmonary conditions, scleroderma, transplant rejection, vasculitis, cytokine storm, and a range of others; 2) neurological diseases and disorders, such as acute trauma or ischemic injury conditions, such as stroke (e.g., ischemic stroke), traumatic brain injury (TBI), spinal cord injury, and neonatal hypoxic ischemia, as well as episodic, chronic, or progressive neurological conditions, such as depression, schizophrenia, epilepsy, Parkinson's Disease, Multiple Sclerosis, Alzheimer's Disease, migraine, ADHD, cerebral palsy, and other conditions; 3) cardiovascular diseases, such as acute myocardial infarction, atherosclerosis, high blood pressure, peripheral vascular disease, and congestive heart failure (CHF); and 4) osteoarthritis.

The following examples are meant to illustrate the invention. They are not meant to limit the invention in any way.

EXAMPLES

Example 1

In some embodiments, in patients with hearts damaged by ischemia (heart attack), permanent impairment of heart function is common. Treatment with mesenchymal stem cells is being tested to evaluate if improvement is possible due to the growth factors, pro-angiogenic factors such as VEGF, and anti-inflammatory factors produced by MSCs. In a study of patients undergoing coronary artery bypass grafting (CABG), MSCs (e.g., bone marrow MSCs) are injected at the time of surgery and the patients receive follow-up evaluation at 6, 12 and 18 months. However, 6 weeks prior to surgery the bone marrow aspirate must be taken to isolate and propagate the MSCs from the patient. At CABG surgery the patients receive 10-20 needle injections into the healthy areas of the heart to deliver 20 million to 200 million MSCs. The use of the cell therapy apparatus or device of the invention would eliminate the waiting period for the growth of the cells, and avoid the many needle sticks into the healthy heart tissue and subsequent risks.

Example 2

In other embodiments, acute respiratory distress syndrome can be caused by many different types of lung injury and the injury causes inflammation. The many anti-inflammatory factors produced by immortalized hMSCs or other stem/progenitor cells can provide reparative effects in the lungs and limit fibrosis and other impairment. Treatment doses for infused MSCs are currently 1, 5 and 10 million per kilogram of body weight (up to 700 million for a typical 70 kg man). The cell therapy apparatus or device of the invention would provide the same potential benefit and without the risks of injecting the cells into the patient.

Example 3

The cell therapy apparatus or device of the invention may be used to improve the transplant outcomes of organ transplants, including lung, heart, kidney, and liver transplants. In some examples, the apparatus or device may be connected to the *in vivo* perfused organ prior to organ harvest from the donor. In other examples, the apparatus or device may be connected to the ex vivo perfused organ after the organ is harvested from the donor, but before it is transplanted to the recipient. In a related example, the apparatus or device is connected to the *ex vivo* perfused organ during organ transport between donor and recipient to improve transplant outcome.

Example 4

The cell therapy apparatus or device of the invention may be used to improve the outcome following trauma-related acute respiratory distress syndrome, such as that which accompanies chest damage related to motor vehicle accidents. The tissue injury may be sterile, but the damage causes an inflammatory response by the body. In this case, the device or apparatus of the invention containing immortalized hMSCs can be connected to the circulatory system to provide anti-inflammatory factors and

improve outcome.

Example 5

The cell therapy apparatus or device containing immortalized hMSCs may be used to reduce bacterial sepsis by delivery of antibacterial prostaglandin E2 (PGE2) and LL-37 peptides. Despite their ability to reduce immune cell activities, MSCs do not increase bacterial sepsis. There are at least two antibacterial factors produced by MSCs, PGE2, and LL-37, which limit bacterial growth and mesenchymal stem cells attenuated myocardial functional depression and reduced systemic and myocardial inflammation during endotoxemia (Weil et al., *Surgery* 148:444-452, 2010; Manukyan et al., *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 300:1506-1514, 2011; Németh et al., *Nat. Med.* 15:42-9, 2009; Krasnodembskaya et al., *Stem Cells* 28:2229-2238, 2010). A device or apparatus of the invention containing immortalized hMSCs can be connected to the circulatory system of a patient having or suspected of having bacterial sepsis to provide antibacterial factors and improve outcome.

Example 6

The apparatus or device containing the immortalized cells can be used to produce conditioned medium that has therapeutic value. The medium is collected after passage through the apparatus and this conditioned medium can be used as is, concentrated, or combined with other factors known to be beneficial. The immortalized cells in the apparatus may be treated with one or more chemicals, bioactive factors, or other cell types that alter their production of molecules from the resident, immortalizd cells such that the conditioned medium now has a new composition. In one example, another cell type such as peripheral blood mononuclear cells are added to the apparatus. The peripheral blood mononuclear cells cause the resident, immortalized cells of the apparatus to produce much more VEGF (vascular endothelial growth factor). In another example, the bioactive factor, such as TGFβ, is inoculated into the apparatus containing the immortalized cells and allowed to interact with the cells for 1 to 36 hrs and the conditioned medium is collected and used for therapeutic purposes or analyzed (see Example 8) to identify the novel molecules produced by the treated cells.

Example 7

The apparatus or device containing the immortalized cells can also be implanted to provide the same benefits or a treatment that may extend over a longer period of time – days, weeks, months, or years. Implantation also provides for an optimal temperature for maintaining the cells in the apparatus or device. For example, the recovery from a traumatic injury may occur over weeks and the implantation of a device or apparatus containing immortalized cells may provide extended benefits not possible with a brief treatment. Further, the cells in the device can be bioengineered to express additional molecules that may provide benefits to specific patients such as diabetic patients, wherein the cells of the apparatus would be engineered to express insulin of a beneficial amount.

Example 8

Beyond the goal of replacing damaged cells within the body, it is clear that cells produce valuable molecules that can be useful in the treatments of many ailments and conditions. The current practice of

injecting cells into patients does not allow us to know which beneficial molecules are produced after injection. The apparatus or device containing the cells allows the cell-produced molecules to be sampled over time or when the cells in the apparatus are treated with another bioactive factor such as TNFα (Perdoni et al. *Stem Cell Research & Therapy* 5:121, 2014; Beltran et al, *J Periodontol.* 86:62-71, 2015) or TGFβ, or other cells are inoculated into the apparatus such as endothelial cells to study molecules produced following cell-cell interactions. The apparatus or device containing the immortalized cells can be used to determine which produced molecules are therapeutically effective. For this, the conditioned medium from the cells in the device are analyzed by separation with gas chromatography followed by mass spectrometry for identifying the individual molecules. The individual molecules can then be removed from the original conditioned medium by chemical means or by immuno-depletion. After depletion of individual molecules, the remaining molecules can be tested *in vitro* or *in vivo* for therapeutic value. By using this approach iteratively, the important active molecules can be determined. Knowing these active molecules, the parameters of the apparatus containing the immortalized cells can be optimized to provide more of these molecules by methods known to one knowledgeable in the field.

OTHER EMBODIMENTS

All publications, patents, and patent applications mentioned in the above specification are hereby incorporated by reference. Various modifications and variations of the described apparatus or device and methods of use of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the art are intended to be within the scope of the invention.

Other embodiments are within the following claims.

CLAIMS

1. An apparatus or device comprising an inlet port and outlet port for connection to an organ, tissue, or subject, wherein said apparatus or device further comprises immortalized cells resident on a matrix in said apparatus or device.

2. The apparatus or device of claim 1, wherein said apparatus or device is for *ex vivo* use or implanted in said subject.

3. The apparatus or device of claim 1, wherein said cells in said apparatus or device produce effluent medium, wherein said effluent medium is conditioned by said cells.

4. The apparatus or device of claim 1, wherein said immortalized cells are stem or progenitor cells.

5. The apparatus or device of claim 1 or 4, wherein said immortalized cells are cells of the lung, heart, liver, bladder, brain, nervous system tissue, blood vessels, skin, eye structures, gut, bone, muscle, ligament, cartilage, esophagus, pancreas, intestines, gallbladder, bile duct, fallopian tubes, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, ureter, urethra, uterus, or fat.

6. The apparatus or device of claim 5, wherein said stem or progenitor cells are embryonic stem cells, induced pluripotent stem cells (IPS), hematopoietic stem cells, intestinal stem cells, osteoblastic stem cells, mesenchymal stem cells (MSCs), multipotent adult progenitor cells (MAPCs), neural stem cells, epithelial stem cells, bone stem cells, cardiac myocyte progenitor stem cells, skin stem cells, skeletal stem cells, muscle stem cells, endothelial stem cells, endothelial progenitor cells, umbilical cord stem cells, adipose stem cells, placental stem cells, placental-derived multipotent stem cells, or liver stem cells.

7. The apparatus or device of claim 6, wherein said mesenchymal stem cells are selected from the group consisting of lung mesenchymal stem cells, Wharton's Jelly mesenchymal stem cells, bone marrow mesenchymal cells, bone marrow stromal cells, umbilical cord mesenchymal cells, spleen mesenchymal cells, adipose derived mesenchymal cells, and pericytes.

8. The apparatus or device of claim 6, wherein said epithelial stem cells are selected from the group consisting of lung epithelial stem cells, breast epithelial stem cells, vascular epithelial stem cells, and intestinal epithelial stem cells.

9. The apparatus or device of claim 6, wherein said skin stem cells are selected from the group consisting of epidermal stem cells, follicular stem cells, and follicle bulge stem cells.

10. The apparatus or device of claim 6, wherein said neural cells are selected from the group consisting of neuronal dopaminergic stem cells and motor neuron stem cells.

11. The apparatus or device of claim 6, wherein said stem or progenitor cells are mesenchymal stem cells (MSCs).

12. The apparatus or device of claim 1, wherein said inlet port or outlet port has one or more of a pre-filter, intermediate filter, or final filter.

13. The apparatus or device of claim 12, wherein one or more of said pre-filter, intermediate filter, and final filter has a filter size in the range of 0.2 – 1 µm.

14. The apparatus or device of claim 13, wherein said pre-filter, intermediate filter, and final filter have a filter size of 1 µm, 0.48 µm, and 0.22 µm, respectively.

15. The apparatus or device of claim 1, wherein said matrix comprises polyvinyl acetal, polylactic acid, polyglycolic acid, poly(lactic/glycolic acid), hollow fiber substrate, titanium, Matrigel, fibronectin, gelatin, laminin, or collagen.

16. The apparatus or device of claim 15, wherein said polyvinyl acetal, polylactic acid, or polyglycolic acid is a foam or woven or nonwoven fabric material.

17. The apparatus or device of claim 1, wherein said apparatus or device is constructed from polycarbonate, polyacrylate, polystyrene, polysulfone, polyester, poly(methyl methacrylate) (PMMA), polymethacrylate (PMA), poly tetrafluoroethylene (PTFE), or fluorinated ethylene propylene copolymer (FEP).

18. The apparatus or device of claim 17, wherein said apparatus or device is constructed from polycarbonate or fluorinated ethylene propylene copolymer (FEP).

19. The apparatus or device of claim 1, wherein said apparatus or device is constructed of flat sheets of material and fused along the edges to create a bag.

20. The apparatus or device of claim 19, wherein said bag contains a matrix.

21. The apparatus or device of claim 1, wherein said apparatus or device comprises a straight, U-shaped, Z-shaped, or multichannel fluid flow path.

22. The apparatus or device of claim 21, wherein said apparatus or device comprises said Z-shaped fluid flow path.

23. The apparatus or device of claim 1, wherein the size of said apparatus or device comprises a length of between 1 to 5 inches, a width of between 1 to 10 inches, and a thickness of between 0.2 to 1.5

inches, particularly wherein said apparatus or device is approximately 3 inches by 5 inches by 0.75 inches.

24. The apparatus or device of claim 1, wherein said apparatus or device comprises a volume of approximately 1 to 100 cm^3.

25. The apparatus or device of claim 1, further comprising a cell culture medium.

26. The apparatus or device of claim 25, wherein said cell culture medium is selected from phosphate-buffered saline (PBS), Dulbecco's Modified Eagle Medium (DMEM), α-Modified Eagles Medium (α-MEM), F-12 medium, or any one or a mixture of the above supplemented with fetal bovine serum (FBS) or human serum, blood, or a blood product.

27. The apparatus or device of claim 26, wherein said cell culture medium is PBS.

28. The apparatus or device of claim 26, wherein the blood product is platelet-rich plasma and/or human serum albumin or recombinant human serum albumin.

29. The apparatus or device of claim 1, further comprising a heating unit that maintains the immortalized cells in said apparatus or device at a temperature in the range of about 20-37°C.

30. The apparatus or device of claim 1, wherein said apparatus or device is connected to an organ or tissue of a subject or to the circulatory system of said subject.

31. The apparatus or device of claim 30, wherein said apparatus or device is connected immediately proximal to the organ or tissue.

32. The apparatus or device of claim 30, wherein said apparatus or device is connected to the circulatory system of said subject.

33. The apparatus or device of claim 30, wherein said organ or tissue is *in vivo* or *ex vivo*.

34. The apparatus or device of any one of claims 30 to 33, wherein said apparatus or device is connected extracorporeally to the subject.

35. The apparatus or device of any one of claims 30 to 33, wherein said apparatus or device is implanted in the subject.

36. The apparatus or device of claim 30, wherein the subject is a transplant recipient, and wherein said apparatus or device is connected to said subject before or after transplantation of an organ or tissue.

37. The apparatus or device of claim 30 or 33, wherein the apparatus or device is connected to said organ or tissue, and wherein said organ or tissue is being prepared for transplantation into a recipient.

38. The apparatus or device of claim 1, wherein said apparatus or device comprising immortalized cells is infused with a bioactive factor or a cell that causes the immortalized cells in the apparatus or device to produce one or more molecules not otherwise made in the apparatus or device.

39. The apparatus or device of claim 38, wherein said bioactive factor is TGFβ or TGFα.

40. The apparatus or device of claim 1, wherein said immortalized cells in said apparatus or device are bioengineered to produce one or more molecules.

41. The apparatus or device of claim 40, wherein said immortalized cells in said apparatus or device are bioengineered to produce insulin.

42. The apparatus or device of claim 1, wherein said apparatus or device comprises a biological sample from a donor or a recipient.

43. The apparatus or device of claim 42, wherein said biological sample comprises one or more cells, fluid, or bioactive factors obtained from said donor or said recipient.

44. The apparatus or device of claim 42 or 43, wherein said biological sample is injected into the apparatus or device.

45. The apparatus or device of claim 30, wherein said organ or tissue is from lung, heart, liver, bladder, brain, blood vessels, skin, eye structures, gut, bone, muscle, ligament, cartilage, esophagus, pancreas, intestines, gallbladder, bile duct, fallopian tubes, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, ureter, urethra, or uterus.

46. The apparatus or device of claim 1, wherein said apparatus or device is connected to a container comprising stem or progenitor cells isolated from a subject.

47. The apparatus or device of claim 46, wherein said apparatus or device promotes proliferation of said stem or progenitor cells.

48. The apparatus or device of claim 1, wherein said subject is a human.

49. The apparatus or device of claim 1, wherein said subject is a non-human mammal.

50. The apparatus or device of claim 1, wherein said immortalized cells are human cells, particularly wherein said human cells are human stem or progenitor cells, such as human MSCs.

51. A method of treating a medical condition comprising establishing a fluid communication between the apparatus or device of claim 1 and an organ, tissue, or subject.

52. The method of claim 51, wherein said subject is a human.

53. The method of claim 51, wherein said subject is a non-human mammal.

54. The method of claim 51, wherein said immortalized cells are human cells, particularly wherein said human cells are human stem or progenitor cells, such as human MSCs.

55. The method of claim 51, wherein said organ or tissue is in a subject.

56. The method of claim 51, wherein said method prepares said organ or tissue for transplantation or improves transplantation outcome.

57. The method of claim 56, wherein said organ or tissue is *in vivo* or *ex vivo*.

58. The method of claim 57, wherein said tissue or organ is or is from lung, heart, liver, bladder, brain, blood vessels, skin, eye structures, gut, bone, muscle, ligament, cartilage, esophagus, pancreas, intestines, gallbladder, bile duct, fallopian tubes, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, ureter, urethra, or uterus.

59. The method of claim 51, wherein said method promotes the proliferation of cells, such as hematopoietic stem cells.

60. A method of treating a medical condition in a subject comprising collecting the effluent medium produced by the cells in the apparatus or device of claim 1 and administering the effluent medium to said subject, wherein said effluent medium is conditioned by the cells.

61. A method for analyzing and identifying for molecules in the effluent medium produced by the cells in the apparatus or device of claim 1 comprising collecting the effluent medium, detecting in the effluent medium one or more molecules produced by the cells, and identifying and isolating the one or more molecules produced by the cells, wherein said effluent medium is conditioned by the cells.

62. The method of claim 60 or 61, further comprising infusing the apparatus or device of claim 1 with a bioactive factor or a cell that causes the immortalized cells in the apparatus or device to produce one or more molecules not otherwise made in the apparatus or device.

63. The apparatus or device of claim 62, wherein said bioactive factor is TGFβ or TGFα.

64. The method of any one of claims 51 to 60, wherein said medical condition is an acute disease, a chronic disease, an autoimmune disease, a skin disease, a neurodegenerative disease, a musculoskeletal disease, a muscle wasting disease, cancer, a vascular or circulatory disease, an inflammatory condition, a cytokine storm condition, an immunological condition, graft versus host disease, a rheumatology disease, cardiac infarction, acute or chronic heart disease, acute or chronic respiratory disease or distress, pulmonary fibrosis, trauma, sepsis, or organ failure.

65. The method of claim 64, wherein said autoimmune disease is Alopecia Areata, Ankylosing Spondylitis, Antiphospholipid Syndrome, Addison's Disease, Hemolytic Anemia, Hepatitis, Behcets Disease, Bullous Pemphigoid, Cardiomyopathy, Celiac Sprue-Dermatitis, Chronic Fatigue Immune Dysfunction Syndrome (CFIDS), Chronic Inflammatory Demyelinating Polyneuropathy, Churg-Strauss Syndrome, Cicatricial Pemphigoid, Limited Scleroderma (CREST Syndrome), Cold Agglutinin Disease, Crohn's Disease, Discoid Lupus, Essential Mixed Cryoglobulinemia, Fibromyalgia-Fibromyositis, Graves' Disease, Guillain-Barré Syndrome , Hashimoto's Thyroiditis, Hypothyroidism, Idiopathic Pulmonary Fibrosis, Idiopathic Thrombocytopenia Purpura (ITP), IgA Nephropathy, insulin dependent diabetes, Juvenile Arthritis, Lichen Planus, Lupus, Ménière's Disease, Mixed Connective Tissue Disease, Multiple Sclerosis, Myasthenia Gravis, Pemphigus Vulgaris, Pernicious Anemia, Polyarteritis Nodosa, Polychondritis, Polyglandular Syndromes, Polymyalgia Rheumatica, Polymyositis and Dermatomyositis, Primary Agammaglobulinemia, Primary Biliary Cirrhosis, Psoriasis, Raynaud's Phenomenon, Reiter's Syndrome, Rheumatic Fever, Rheumatoid Arthritis, Sarcoidosis, Scleroderma, Sjögren's Syndrome, Stiff-Man Syndrome, Takayasu Arteritis, Temporal Arteritis/Giant Cell Arteritis, Ulcerative Colitis, Uveitis, Vasculitis, Vitiligo, or Wegener's Granulomatosis.

66. The method of claim 65, wherein said autoimmune disease is insulin dependent diabetes.

67. The method of any one of claims 51 to 60, wherein said neurodegenerative disease is a neurological disorder selected from the group consisting of Parkinson’s disease, Alzheimer’s disease, amyotrophic lateral sclerosis (ALS), Huntington’s disease, cerebral stroke, traumatic brain injury, and spinal cord injury.

68. The method of any one of claims 51 to 60, wherein said cancer is bladder cancer, pancreatic cancer, cervical cancer, lung cancer, liver cancer, ovarian cancer, colon cancer, stomach cancer, virally induced cancer, neuroblastoma, breast cancer, prostate cancer, renal cancer, leukemia, sarcomas, myeloma, or a carcinoma.

69. The method of claim 68, wherein said method treats cachexia, sarcopenia, reduces chemotherapy-induced inflammation, reduces sepsis, or reduces cancer cell proliferation.

70. The method of any one of claims 51 to 60, wherein said inflammatory condition is osteoarthritis, transplantation related inflammation, cytokine storm, ulcerative colitis, Crohn's disease, proctitis, microscopic colitis, allergic eosinophilic gastroenteritis, food allergies, pill induced esophagitis, celiac

disease, recurrent polyps, hemorrhoids, bacterial sepsis, sterile inflammation, an immunological condition, graft versus host disease, or trauma related tissue injury.

71. A kit comprising the apparatus or device of claim 1 and a cell culture medium or PBS.

72. The kit of claim 71, wherein said cell culture medium is in said apparatus or device.

73. The kit of claim 71, wherein said cell culture medium is in a container in said kit.

74. The kit of any one of claims 71 to 73, further comprising a heating unit.

75. The kit of claim 71, wherein immortalized cells are present in said apparatus or device, or are present in a container within said kit.

76. The kit of claim 71, further comprising a reservoir for collecting effluent medium, wherein said effluent medium can be used for analysis, research, or therapeutic use.

77. The apparatus or device of any one of claims 1 to 14, wherein said matrix comprises polyvinyl acetal, polylactic acid, polyglycolic acid, poly(lactic/glycolic acid), hollow fiber substrate, titanium, Matrigel, fibronectin, gelatin, laminin, or collagen.

78. The apparatus or device of claim 77, wherein said polyvinyl acetal, polylactic acid, or polyglycolic acid is a foam or woven or nonwoven fabric material.

79. The apparatus or device of any one of claims 1 to 14, 77, and 78, wherein said apparatus or device is constructed from polycarbonate, polyacrylate, polystyrene, polysulfone, polyester, poly(methyl methacrylate) (PMMA), polymethacrylate (PMA), poly tetrafluoroethylene (PTFE), or fluorinated ethylene propylene copolymer (FEP).

80. The apparatus or device of claim 79, wherein said apparatus or device is constructed from polycarbonate or fluorinated ethylene propylene copolymer (FEP).

81. The apparatus or device of any one of claims 1 to 14 and 77 to 80, wherein said apparatus or device is constructed of flat sheets of material and fused along the edges to create a bag.

82. The apparatus or device of claim 81, wherein said bag contains a matrix.

83. The apparatus or device of any one of claims 1 to 14 and 77 to 82, wherein said apparatus or device comprises a straight, U-shaped, Z-shaped, or multichannel fluid flow path.

84. The apparatus or device of claim 83, wherein said apparatus or device comprises said Z-shaped fluid flow path.

85. The apparatus or device of any one of claims 1 to 14 and 77 to 84, wherein the size of said apparatus or device comprises a length of between 1 to 5 inches, a width of between 1 to 10 inches, and a thickness of between 0.2 to 1.5 inches, particularly wherein said apparatus or device is approximately 3 inches by 5 inches by 0.75 inches.

86. The apparatus or device of any one of claims 1 to 14 and 77 to 85, wherein said apparatus or device comprises a volume of approximately 1 to 100 cm^3.

87. The apparatus or device of any one of claims 1 to 14 and 77 to 86, further comprising a cell culture medium.

88. The apparatus or device of claim 87, wherein said cell culture medium is selected from phosphate-buffered saline (PBS), Dulbecco's Modified Eagle Medium (DMEM), α-Modified Eagles Medium (α-MEM), F-12 medium, or any one or a mixture of the above supplemented with fetal bovine serum (FBS) or human serum, blood, or a blood product.

89. The apparatus or device of claim 88, wherein said cell culture medium is PBS.

90. The apparatus or device of claim 88, wherein the blood product is platelet-rich plasma and/or human serum albumin or recombinant human serum albumin.

91. The apparatus or device of any one of claims 1 to 14 and 77 to 90, further comprising a heating unit that maintains the immortalized cells in said apparatus or device at a temperature in the range of about 20-37°C.

92. The apparatus or device of any one of claims 1 to 14 and 77 to 91, wherein said apparatus or device is connected to an organ or tissue of a subject or to the circulatory system of said subject.

93. The apparatus or device of claim 92, wherein said apparatus or device is connected immediately proximal to the organ or tissue.

94. The apparatus or device of claim 92, wherein said apparatus or device is connected to the circulatory system of said subject.

95. The apparatus or device of claim 92 or 93, wherein said organ or tissue is *in vivo* or *ex vivo*.

96. The apparatus or device of any one of claims 92 to 95, wherein said apparatus or device is connected extracorporeally to the subject.

97. The apparatus or device of any one of claims 92 to 95, wherein said apparatus or device is implanted in the subject.

98. The apparatus or device of any one of claims 92 to 97, wherein the subject is a transplant recipient, and wherein said apparatus or device is connected to said subject before or after transplantation of an organ or tissue.

99. The apparatus or device of any one of claims 92, 93, and 95, wherein the apparatus or device is connected to said organ or tissue, and wherein said organ or tissue is being prepared for transplantation into a recipient.

100. The apparatus or device of any one of claims 1 to 14 and 77 to 99, wherein said apparatus or device comprising immortalized cells is infused with a bioactive factor or a cell that causes the immortalized cells in the apparatus or device to produce one or more molecules not otherwise made in the apparatus or device.

101. The apparatus or device of claim 100, wherein said bioactive factor is TGFβ or TGFα.

102. The apparatus or device any one of claims 1 to 14 and 77 to 101, wherein said immortalized cells in said apparatus or device are bioengineered to produce one or more molecules.

103. The apparatus or device of claim 102, wherein said immortalized cells in said apparatus or device are bioengineered to produce insulin.

104. The apparatus or device of any one of claims 1 to 14 and 77 to 103, wherein said apparatus or device comprises a biological sample from a donor or a recipient.

105. The apparatus or device of claim 104, wherein said biological sample comprises one or more cells, fluid, or bioactive factors obtained from said donor or said recipient.

106. The apparatus or device of claim 104 or 105, wherein said biological sample is injected into the apparatus or device.

107. The apparatus or device of any one of claims 92 to 106, wherein said organ or tissue is from lung, heart, liver, bladder, brain, blood vessels, skin, eye structures, gut, bone, muscle, ligament, cartilage, esophagus, pancreas, intestines, gallbladder, bile duct, fallopian tubes, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, ureter, urethra, or uterus.

108. The apparatus or device of any one of claims 1 to 14 and 77 to 91, wherein said apparatus or device is connected to a container comprising stem or progenitor cells isolated from a subject.

109. The apparatus or device of claim 108, wherein said apparatus or device promotes proliferation of said stem or progenitor cells.

110. The apparatus or device of any one of claims 1 to 14 and 77 to 109, wherein said subject is a human.

111. The apparatus or device of any one of claims 1 to 14 and 77 to 109, wherein said subject is a non-human mammal.

112. The apparatus or device of any one of claims 1 to 14 and 77 to 109, wherein said immortalized cells are human cells, particularly wherein said human cells are human stem or progenitor cells, such as human MSCs.

113. A method of treating a medical condition comprising establishing a fluid communication between the apparatus or device of any one of claims 1 to 14 and 77-112 and an organ, tissue, or subject.

114. The method of claim 113, wherein said subject is a human.

115. The method of claim 113, wherein said subject is a non-human mammal.

116. The method of claim 113, wherein said immortalized cells are human cells, particularly wherein said human cells are human stem or progenitor cells, such as human MSCs.

117. The method of claim 113, wherein said organ or tissue is in a subject.

118. The method of any one of claims 113 to 117, wherein said method prepares said organ or tissue for transplantation or improves transplantation outcome.

119. The method of any one of claims 113 to 118, wherein said organ or tissue is *in vivo* or *ex vivo*.

120. The method of claim 119, wherein said tissue or organ is or is from lung, heart, liver, bladder, brain, blood vessels, skin, eye structures, gut, bone, muscle, ligament, cartilage, esophagus, pancreas, intestines, gallbladder, bile duct, fallopian tubes, ovaries, prostate, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, ureter, urethra, or uterus.

121. The method of any one of claims 113 to 120, wherein said method promotes the proliferation of cells, such as hematopoietic stem cells.

122. A method of treating a medical condition in a subject comprising collecting the effluent medium produced by the cells in the apparatus or device of any one of claims 1 to 14 and 77 to 112 and

administering the effluent medium to said subject, wherein said effluent medium is conditioned by the cells.

123. A method for analyzing and identifying for molecules in the effluent medium produced by the cells in the apparatus or device of any one of claims 1 to 14 and 77 to 112 comprising collecting the effluent medium, detecting in the effluent medium one or more molecules produced by the cells, and identifying and isolating the one or more molecules produced by the cells, wherein said effluent medium is conditioned by the cells.

124. The method of claim 122 or 123, further comprising infusing the apparatus or device of claim 1 with a bioactive factor or a cell that causes the immortalized cells in the apparatus or device to produce one or more molecules not otherwise made in the apparatus or device.

125. The apparatus or device of claim 124, wherein said bioactive factor is TGFβ or TGFα.

126. The method of claim 113 to 122, wherein said medical condition is an acute disease, a chronic disease, an autoimmune disease, a skin disease, a neurodegenerative disease, a musculoskeletal disease, a muscle wasting disease, cancer, a vascular or circulatory disease, an inflammatory condition, a cytokine storm condition, an immunological condition, graft versus host disease, a rheumatology disease, cardiac infarction, acute or chronic heart disease, acute or chronic respiratory disease or distress, pulmonary fibrosis, trauma, sepsis, or organ failure.

127. The method of any one of claims 113 to 122, wherein said autoimmune disease is Alopecia Areata, Ankylosing Spondylitis, Antiphospholipid Syndrome, Addison's Disease, Hemolytic Anemia, Hepatitis, Behcets Disease, Bullous Pemphigoid, Cardiomyopathy, Celiac Sprue-Dermatitis, Chronic Fatigue Immune Dysfunction Syndrome (CFIDS), Chronic Inflammatory Demyelinating Polyneuropathy, Churg-Strauss Syndrome, Cicatricial Pemphigoid, Limited Scleroderma (CREST Syndrome), Cold Agglutinin Disease, Crohn's Disease, Discoid Lupus, Essential Mixed Cryoglobulinemia, Fibromyalgia-Fibromyositis, Graves' Disease, Guillain-Barré Syndrome , Hashimoto's Thyroiditis, Hypothyroidism, Idiopathic Pulmonary Fibrosis, Idiopathic Thrombocytopenia Purpura (ITP), IgA Nephropathy, insulin dependent diabetes, Juvenile Arthritis, Lichen Planus, Lupus, Ménière's Disease, Mixed Connective Tissue Disease, Multiple Sclerosis, Myasthenia Gravis, Pemphigus Vulgaris, Pernicious Anemia, Polyarteritis Nodosa, Polychondritis, Polyglandular Syndromes, Polymyalgia Rheumatica, Polymyositis and Dermatomyositis, Primary Agammaglobulinemia, Primary Biliary Cirrhosis, Psoriasis, Raynaud's Phenomenon, Reiter's Syndrome, Rheumatic Fever, Rheumatoid Arthritis, Sarcoidosis, Scleroderma, Sjögren's Syndrome, Stiff-Man Syndrome, Takayasu Arteritis, Temporal Arteritis/Giant Cell Arteritis, Ulcerative Colitis, Uveitis, Vasculitis, Vitiligo, or Wegener's Granulomatosis.

128. The method of claim 127, wherein said autoimmune disease is insulin dependent diabetes.

129. The method of any one of claims 113 to 122, wherein said neurodegenerative disease is a neurological disorder selected from the group consisting of Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Huntington's disease, cerebral stroke, traumatic brain injury, and spinal cord injury.

130. The method of any one of claims 113 to 122, wherein said cancer is bladder cancer, pancreatic cancer, cervical cancer, lung cancer, liver cancer, ovarian cancer, colon cancer, stomach cancer, virally induced cancer, neuroblastoma, breast cancer, prostate cancer, renal cancer, leukemia, sarcomas, myeloma, or a carcinoma.

131. The method of claim 130, wherein said method treats cachexia, sarcopenia, reduces chemotherapy-induced inflammation, reduces sepsis, or reduces cancer cell proliferation.

132. The method of any one of claims 113 to 122, wherein said inflammatory condition is osteoarthritis, transplantation related inflammation, cytokine storm, ulcerative colitis, Crohn's disease, proctitis, microscopic colitis, allergic eosinophilic gastroenteritis, food allergies, pill induced esophagitis, celiac disease, recurrent polyps, hemorrhoids, bacterial sepsis, sterile inflammation, an immunological condition, graft versus host disease, or trauma related tissue injury.

133. A kit comprising the apparatus or device of any one of claims 1 to 14 and 77 to 112 and a cell culture medium or PBS.

134. The kit of claim 133, wherein said cell culture medium is in said apparatus or device.

135. The kit of claim 133, wherein said cell culture medium is in a container in said kit.

136. The kit of any one of claims 133 to 135, further comprising a heating unit.

137. The kit of any one of claims 133 to 136, wherein immortalized cells are present in said apparatus or device, or are present in a container within said kit.

138. The kit of any one of claims 133 to 137, further comprising a reservoir for collecting effluent medium, wherein said effluent medium can be used for analysis, research, or therapeutic use.

139. The method of claim 100 or 124, wherein said cell that causes the immortalized cells in the apparatus or device to produce one or more molecules not otherwise made in the apparatus or device is a peripheral blood mononuclear cell.

140. The method of claim 38, wherein said cell that causes the immortalized cells in the apparatus or device to produce one or more molecules not otherwise made in the apparatus or device is a peripheral blood mononuclear cell.

141. The method of claim 62, wherein said cell that causes the immortalized cells in the apparatus or device to produce one or more molecules not otherwise made in the apparatus or device is a peripheral blood mononuclear cell.

FIG. 1

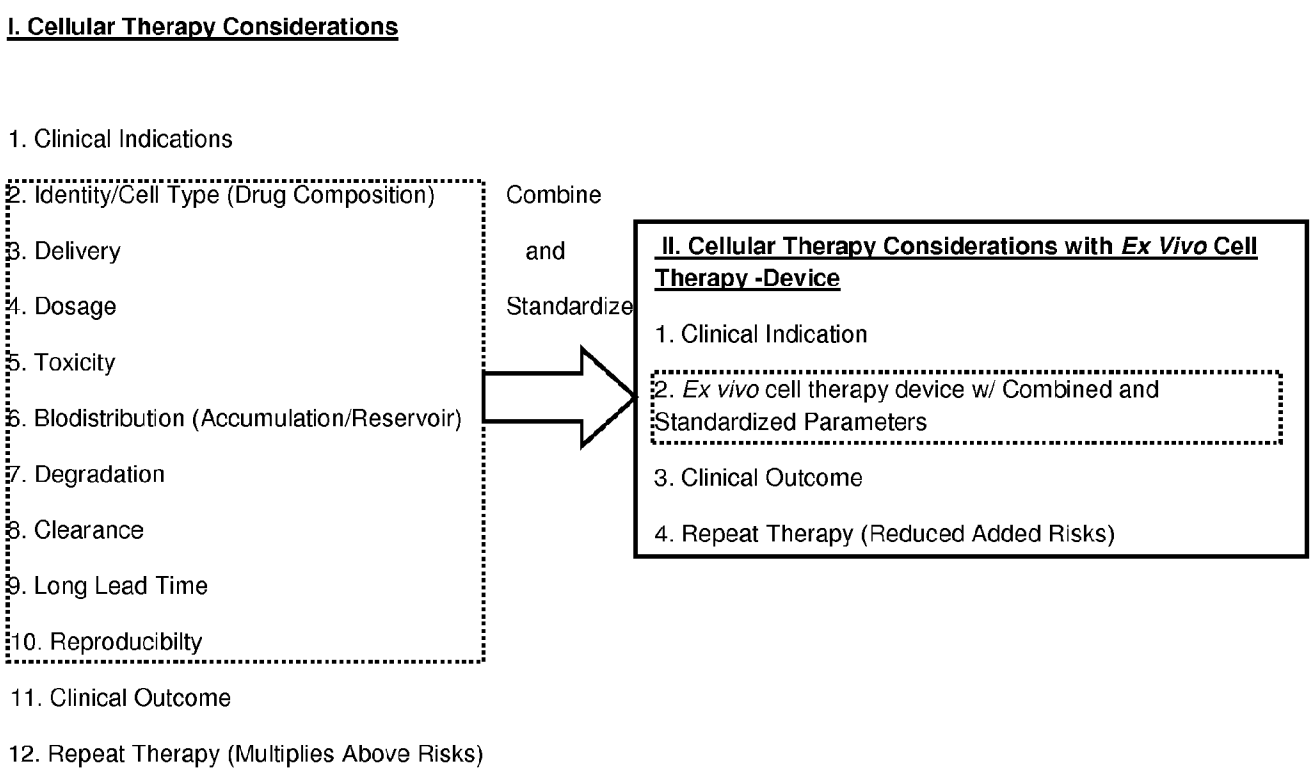

I. Cellular Therapy Considerations
1. Clinical Indications
2. Identity/Cell Type (Drug Composition)
3. Delivery
4. Dosage
5. Toxicity
6. Blodistribution (Accumulation/Reservoir)
7. Degradation
8. Clearance
9. Long Lead Time
10. Reproducibilty
11. Clinical Outcome
12. Repeat Therapy (Multiplies Above Risks)
Combine
and
Standardize
II. Cellular Therapy Considerations with Ex Vivo Cell Therapy -Device
1. Clinical Indication
2. Ex vivo cell therapy device w/ Combined and Standardized Parameters
3. Clinical Outcome
4. Repeat Therapy (Reduced Added Risks)

FIG. 2A

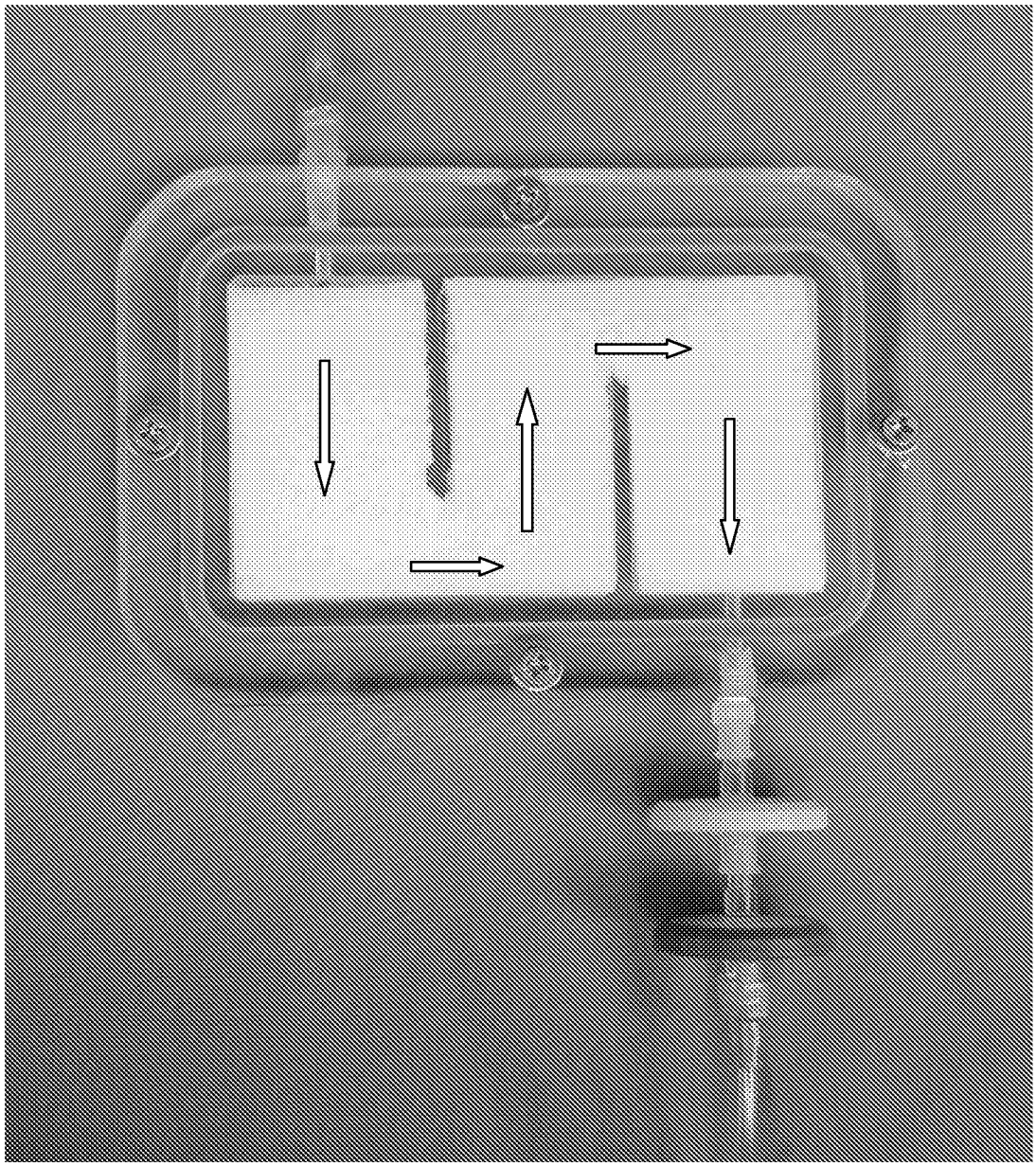


FIG. 2B

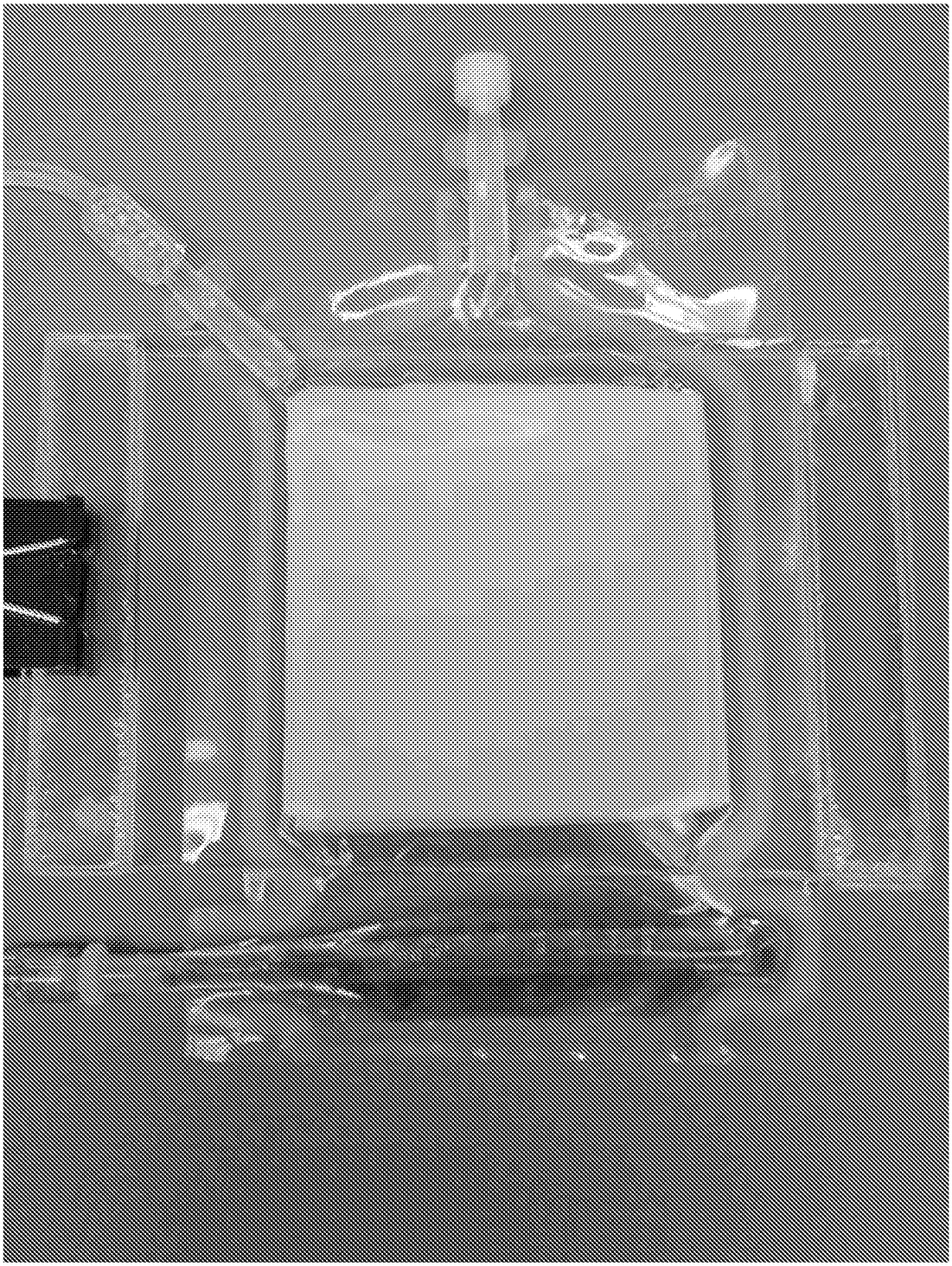


FIG. 3A

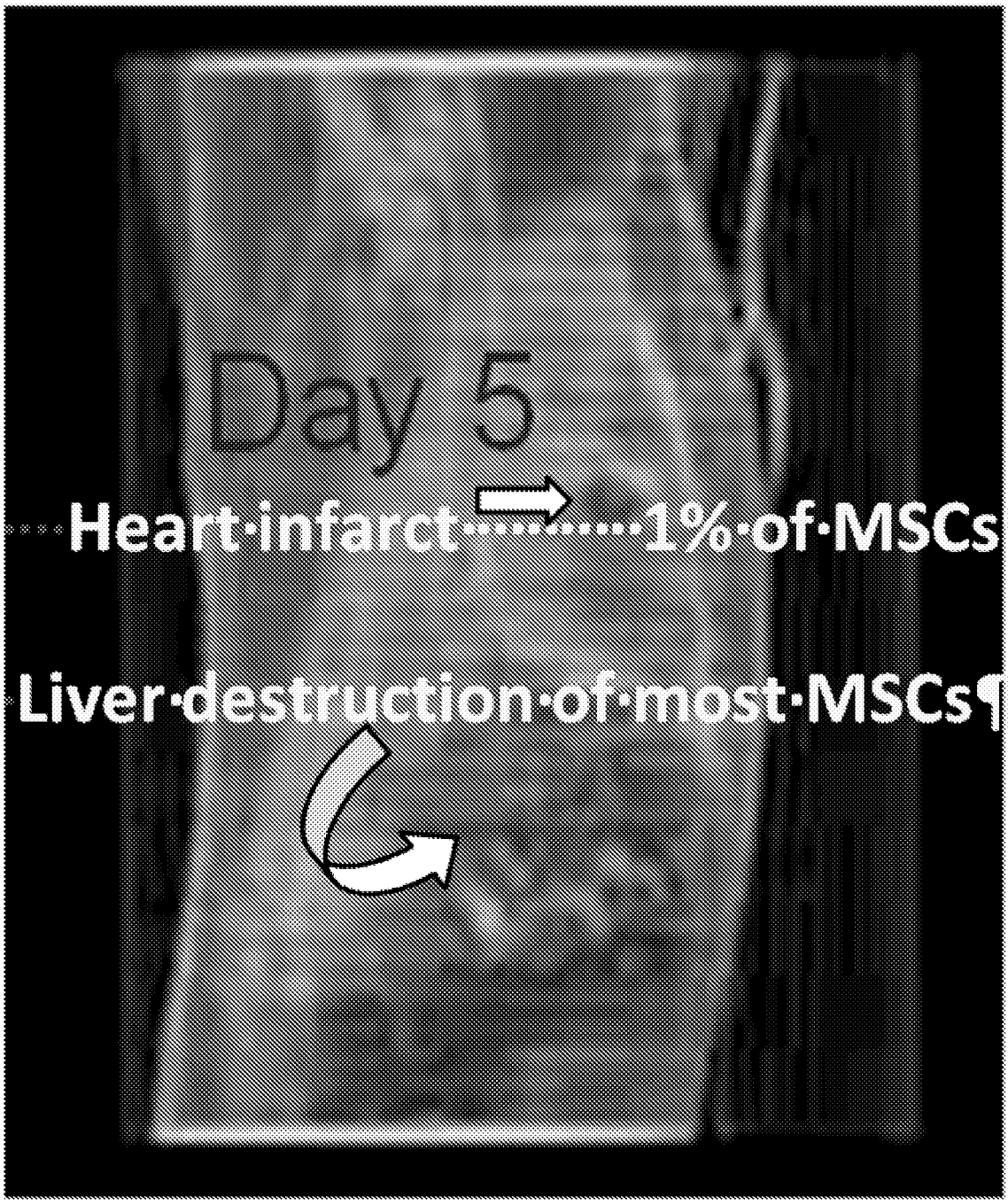


FIG. 3B

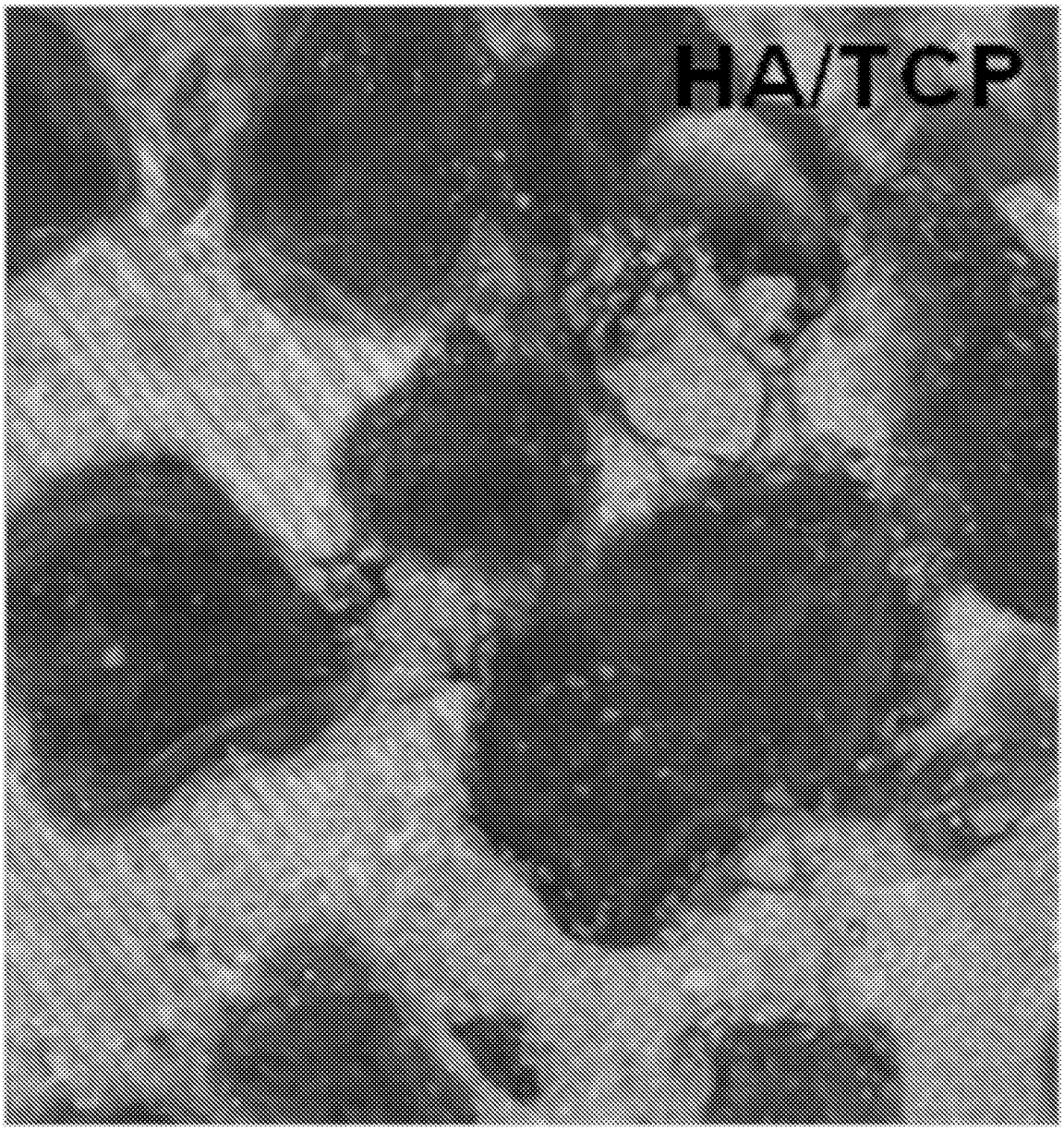


FIG. 4

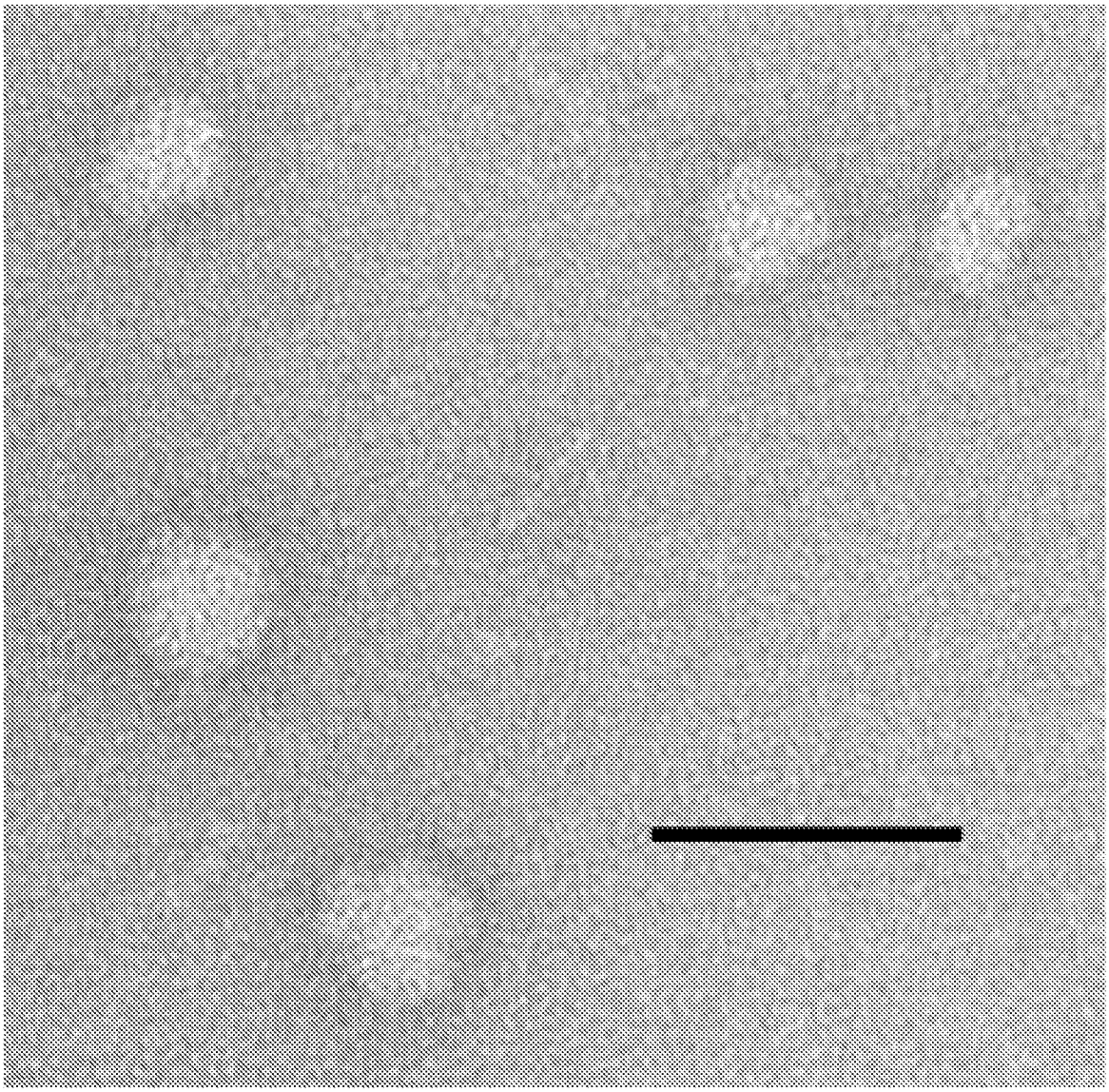
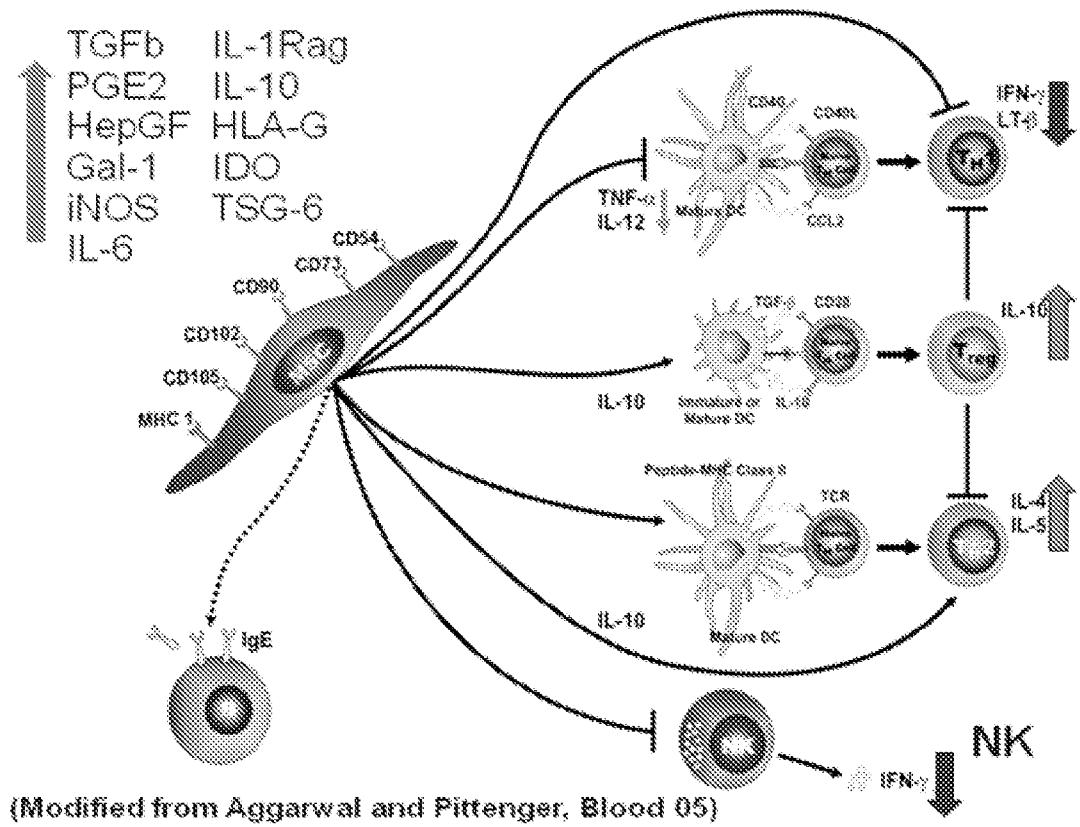


FIG. 5



(Modified from Aggarwal and Pittenger, Blood 05)

FIG. 6

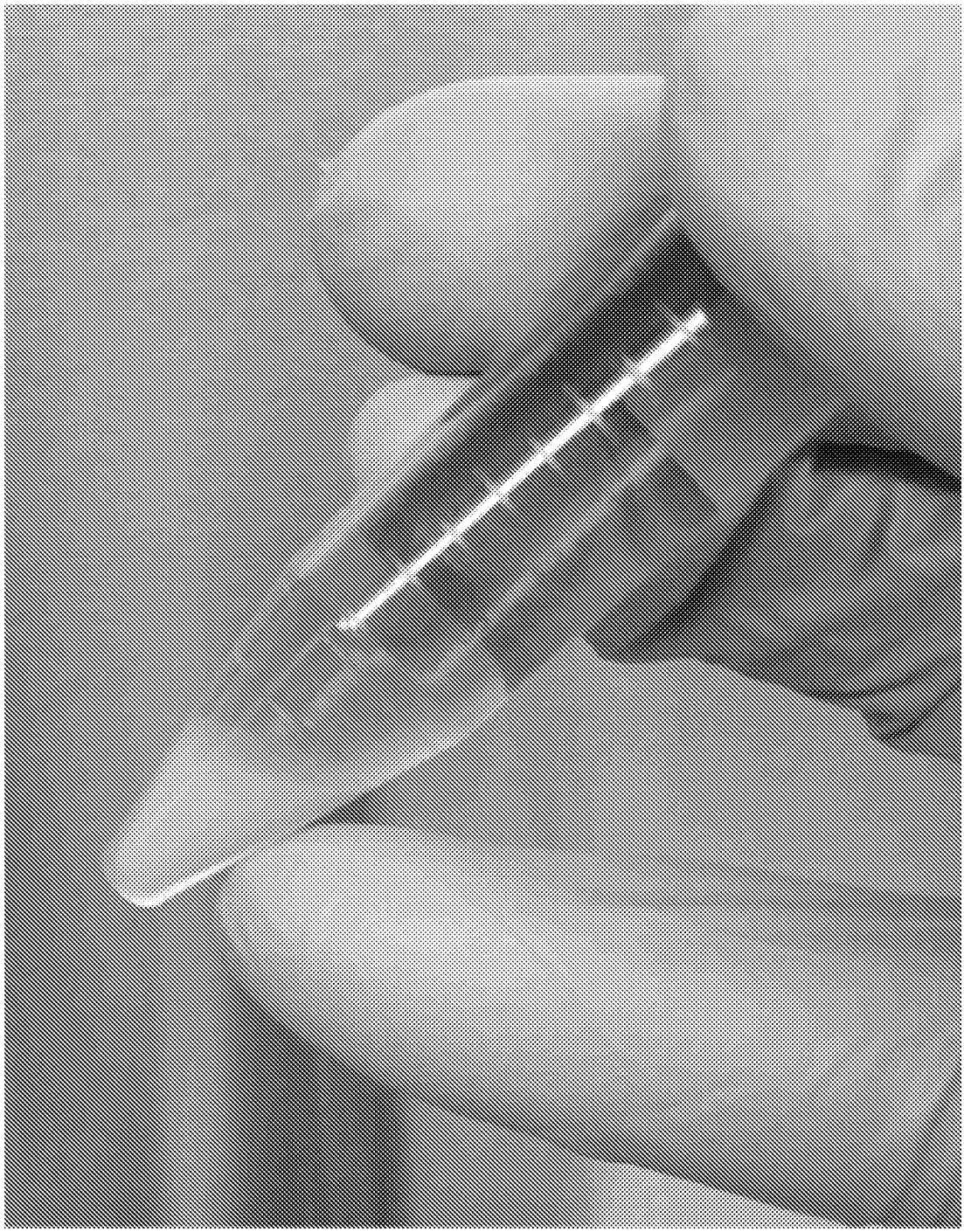
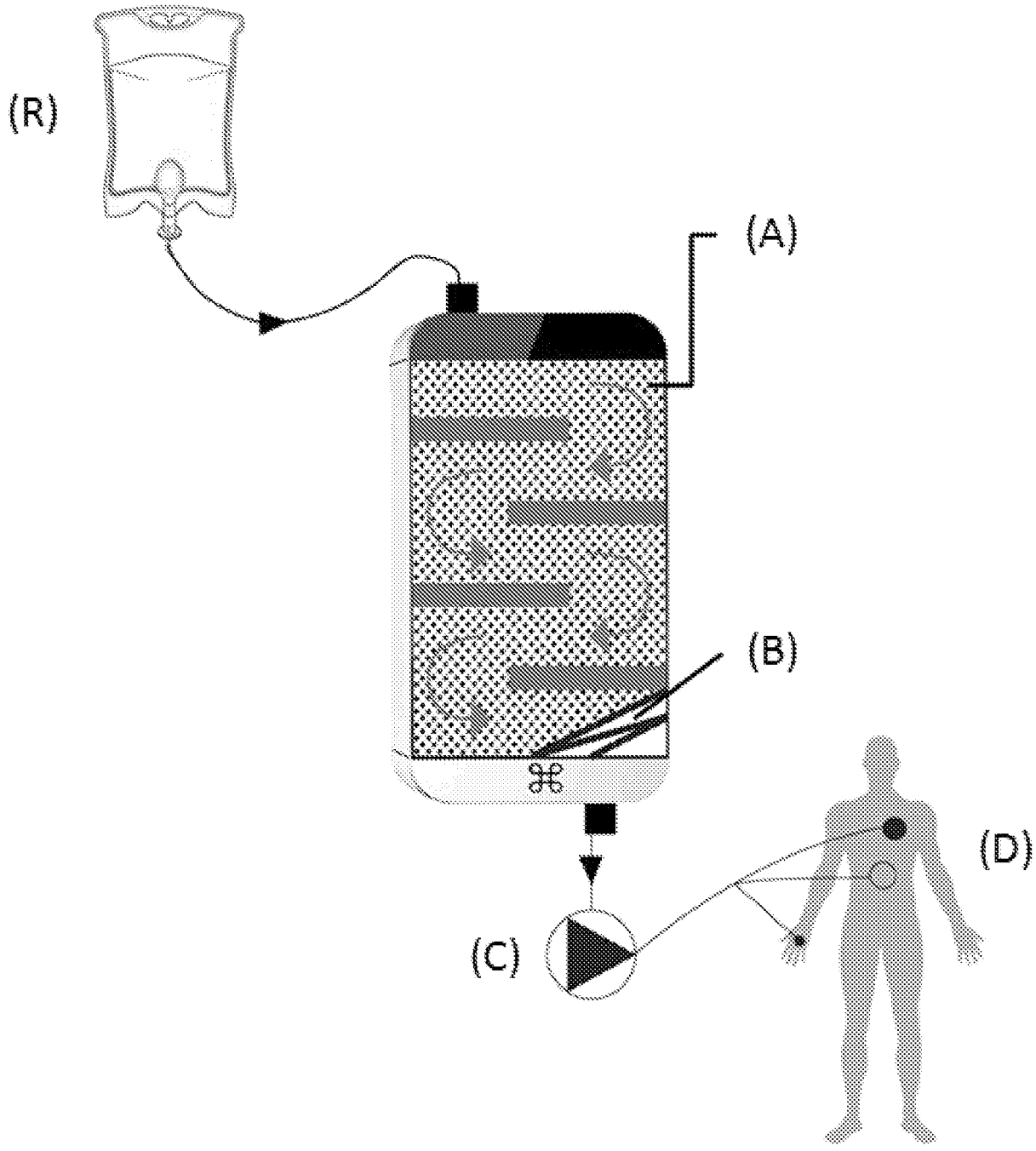


FIG. 7



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/25973

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 9/00, A61K 48/00, C12N 5/0797, C12N 5/071 (2015.01)
CPC - A61K 9/00, A61K 9/0024, A61K 48/00, C12N 5/06, C12N 2502/03
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)
IPC(8)- A61K 9/00, A61K 48/00, C12N 5/0797, C12N 5/071 (2015.01);
CPC- A61K 9/00, A61K 9/0024, A61K 48/00, C12N 5/06, C12N 2502/03

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC- 424/93.2, 424/422, 424/425, 435/325, 435/366, 435/378, 435/FOR214;
Patents and NPL (classification, keyword; search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Pub West (US EP JP WO), Pat Base (AU BE BR CA CH CN DE DK EP ES FI FR GB IN JP KR SE TH TW US WO), Google Patent, Google Scholar, Free Patents Online; search terms: immortalize, cell, tissue, organ, stem, progenitor, matrix, culture, medium, saline, TGF, transform, growth, inject, implant, inlet, outlet, flow, port, bag

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2010/0303774 A1 (HEDRICK et al.) 02 December 2010 (02.12.2010), Fig. 1; para [0009]-[0011], [0029]-[0035], [0050], [0051], [0054]-[0057], [0060], [0061], [0064]-[0066], [0072], [0076], [0077], [0079], [0082], [0101], [0109]-[0113], [0117], [0129], [0155], [0166], [0187], [0205]	1-63, 71-76, 140, 141
Y	US 2005/0025838 A1 (BADYLAK) 03 February 2005 (03.02.2005), para [0009], [0030], [0033], [0039], [0041], [0042], [0047], [0048], [0059], [0061], [0063], [0072], [0077], [0078], [0088]	1-63, 71-76, 140, 141
Y	US 2008/0254007 A1 (SESHI) 16 October 2008 (16.10.2008), para [0021]-[0350]	1-63, 71-76, 140, 141
Y	US 2008/0014181 A1 (ARIFF et al.) 17 January 2008 (17.01.2008), para [0019]-[0140]	1-63, 71-76, 140, 141
Y	US 2005/0014255 A1 (TANG et al.) 20 January 2005 (20.01.2005), para [0009]-[0135]	1-63, 71-76, 140, 141

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
06 June 2015 (06.06.2015)	02 JUL 2015
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)