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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 December 2020 (24.12.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/257720 A1

(51) International Patent Classification:

A61K 9/127 (2006.01)	*A61P 7/00* (2006.01)
A61K 9/50 (2006.01)	*A61P 9/00* (2006.01)
A61K 35/00 (2006.01)	*A61P 9/10* (2006.01)
A61K 35/50 (2015.01)	*A61P 11/00* (2006.01)
A61K 45/06 (2006.01)	*A61P 13/12* (2006.01)
A61K 51/12 (2006.01)	*A61P 17/00* (2006.01)
A61P 1/00 (2006.01)	*A61P 27/02* (2006.01)
A61P 1/16 (2006.01)	*C12N 15/113* (2010.01)
A61P 3/00 (2006.01)	*C12N 15/88* (2006.01)

(21) International Application Number:
PCT/US2020/038828

(22) International Filing Date:
19 June 2020 (19.06.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

62/863,767	19 June 2019 (19.06.2019)	US
62/891,700	26 August 2019 (26.08.2019)	US
62/905,117	24 September 2019 (24.09.2019)	US
62/924,147	21 October 2019 (21.10.2019)	US

(71) Applicant: **CELULARITY INC.** [US/US]; 33 Technology Drive, Warren, New Jersey 07059 (US).

(72) Inventors: **HARIRI, Robert J.**; c/o Celularity Inc., 33 Technology Drive, Warren, New Jersey 07059 (US). **ZHANG, Xiaokui**; c/o Celularity Inc., 33 Technology Drive, Warren, New Jersey 07059 (US). **YE, Qian**; c/o Celularity Inc., 33 Technology Drive, Warren, New Jersey 07059 (US). **HE, Shuyang**; c/o Celularity Inc., 33 Technology Drive, Warren, New Jersey 07059 (US). **HARIRI, Haley**; c/o Celularity Inc., 33 Technology Drive, Warren, New Jersey 07059 (US). **SHAH, Navjot**; c/o Celularity Inc., 33 Technology Drive, Warren, New Jersey 07059 (US). **SOMANCHI, Srinivas**; c/o Celularity Inc., 33 Technology Drive, Warren, New Jersey 07059 (US). **STOUT, Bhavani**;

(54) Title: EXOSOMES FOR DISEASE TREATMENT

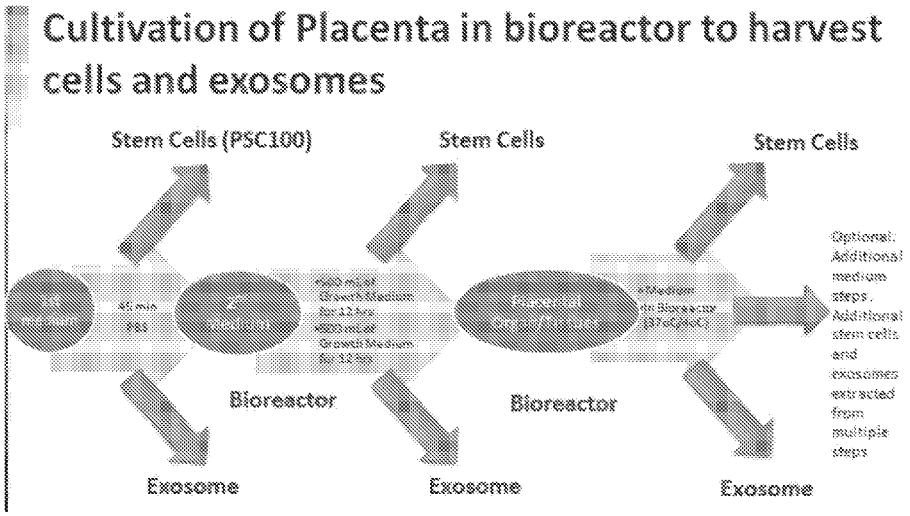


FIG. 1

(57) Abstract: The present invention provides method of treating diseases, disorders and conditions in a human subject comprising administering to the subject a population of exosomes or a composition comprising a population of exosomes, wherein said population of exosomes is positive for CD1c, CD20, CD24, CD25, CD29, CD2, CD3, CD8, CD9, CD11c, CD14, CD19, CD31, CD40, CD41b, CD42a, CD44, CD45, CD49e, CD4, CD56, CD62P, CD63, CD69, CD81, CD86, CD105, CD133-1, CD142, CD146, CD209, CD326, HLA-ABC, HLA-DRDPDQ, MCSP, ROR1, SSEA-4, or combinations thereof. Such diseases, disorders and conditions include lung, liver, central nervous system, kidney, cardiovascular, gastrointestinal, spleen, eye, systemic and ageing associated diseases, disorders, and conditions.

WO 2020/257720 A1

[Continued on next page]

c/o Celularity Inc., 33 Techhnology Drive, Warren, New Jersey 07059 (US).

(74) **Agent: KNUDSEN, Geoffry, T.**; Celularity, Inc., 33 Technology Drive, Warren, New Jersey 07059 (US).

(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, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, 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, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, 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, 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:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

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

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

EXOSOMES FOR DISEASE TREATMENT

[0001] This application claims benefit of U.S. Provisional Patent Application Nos. 62/863,767 filed June 19, 2019; 62/891,700 filed August 26, 2019; 62/905,117 filed September 24, 2019, and 62/924,147 filed October 21, 2019, the disclosures of which are incorporated by reference herein in their entireties.

1. FIELD OF THE INVENTION

[0002] Methods of using exosomes to treat diseases or conditions in a patient and specific exosome populations as well as characteristics of said populations which are particularly effective for such treatment are taught in the subject application.

2. BACKGROUND OF THE INVENTION

[0003] Exosomes are nano-sized bi-lipid membrane vesicles secreted from living cells, which play important functions in cell-cell communications. During human pregnancy, the placenta plays a central role in regulating physiological homeostasis and supporting fetal development. It is known that extracellular vesicles and exosomes secreted by placenta contribute to the communication between placenta and maternal tissues to maintain maternal-fetal tolerance. Exosomes contain active biologics including lipids, cytokines, microRNA, mRNA and DNA, as well as, proteins, which can be presented on the surface of the exosomes. Exosomes are thought to be useful for many therapeutic approaches including immune modulation, the promotion of angiogenesis, and for the delivery of medicaments. The need for more approaches that allow for the isolation of large quantities of exosomes is manifest.

3. SUMMARY

[0004] Aspects of the present invention concern methods to produce, isolate, and characterize exosomes from a cultivated placenta or a portion thereof. The present invention also provides methods of treating diseases or disorders in a subject with populations of exosomes; particularly populations of exosomes produced as described herein or having characteristic described herein.

[0005] The exosomes described herein comprise particular markers. Such markers can, for example, be useful in the identification of the exosomes and for distinguishing them from other exosomes, *e.g.*, exosomes not derived from placenta. In certain embodiments, such exosomes are positive for one or more markers, *e.g.*, as determinable by flow cytometry, for example, by fluorescence-activated cell sorting (FACS). In addition, the exosomes provided herein can be identified based on the absence of certain markers. Determination of the presence or absence of such markers can be accomplished using methods known in the art, *e.g.*, fluorescence-activated cell sorting (FACS).

[0006] The present invention provides methods of treating a disease, disorder or condition in a subject comprising administering to the subject a population of exosomes or a composition comprising a population of exosomes, wherein said population of exosomes is positive for CD1c, CD20, CD24, CD25, CD29, CD2, CD3, CD8, CD9, CD11c, CD14, CD19, CD31, CD40, CD41b, CD42a, CD44, CD45, CD49e, CD4, CD56, CD62P, CD63, CD69, CD81, CD86, CD105, CD133-1, CD142, CD146, CD209, CD326, HLA-ABC, HLA-DRDPDQ, MCSP, ROR1, SSEA-4, or combinations thereof.

[0007] In some embodiments said population of exosomes is positive for CD1c, CD20, CD24, CD25, CD29, CD2, CD3, CD8, CD9, CD11c, CD14, CD19, CD31, CD40, CD41b, CD42a, CD44, CD45, CD49e, CD4, CD56, CD62P, CD63, CD69, CD81, CD86, CD105, CD133-1, CD142, CD146, CD209, CD326, HLA-ABC, HLA-DRDPDQ, MCSP, ROR1, and SSEA-4. In some embodiments said population of exosomes is positive for 2, 3, 4, 5, 6, 7, 8, 9, 10, or more markers selected from the group consisting of CD1c, CD20, CD24, CD25, CD29, CD2, CD3, CD8, CD9, CD11c, CD14, CD19, CD31, CD40, CD41b, CD42a, CD44, CD45, CD49e, CD4, CD56, CD62P, CD63, CD69, CD81, CD86, CD105, CD133-1, CD142, CD146, CD209, CD326, HLA-ABC, HLA-DRDPDQ, MCSP, ROR1, and SSEA-4.

[0008] In some embodiments said population of exosomes is positive for CD9, CD29, CD42a, CD62P, CD63, CD81, CD133-1, CD146, HLA-DRP, or combinations thereof. In some embodiments said population of exosomes is positive for CD9, CD29, CD42a, CD62P, CD63, CD81, CD133-1, CD146, and HLA-DRP. In some embodiments said population of exosomes is positive for 2, 3, 4, 5, 6, 7, 8, 9, 10, or more markers selected from the group consisting of CD9, CD29, CD42a, CD62P, CD63, CD81, CD133-1, CD146, and HLA-DRP.

[0009] In some embodiments said population of exosomes is CD3-, CD11b-, CD14-, CD19-, CD33-, CD192-, HLA-A-, HLA-B-, HLA-C-, HLA-DR-, CD11c- or CD34 . In some embodiments said population of exosomes is CD3-, CD11b-, CD14-, CD19-, CD33-, CD192-, HLA-A-, HLA-B-, HLA-C-, HLA-DR-, CD11c- and CD34-.

[0010] In some embodiments said population of exosomes comprise non-coding RNA molecules. In some embodiments said non-coding RNA molecules are microRNAs. In some embodiments said microRNAs are selected from the group consisting of the microRNAs in Table 7, and combinations thereof. In some embodiments said microRNAs are selected from the group consisting of hsa-mir-26b, hsa-miR-26b-5p, hsa-mir-26a-2, hsa-mir-26a-1, hsa-miR-26a-5p, hsa-mir-30d, hsa-miR-30d-5p, hsa-mir-100, hsa-miR-100-5p, hsa-mir-21, hsa-miR-21-5p, hsa-mir-22, hsa-miR-22-3p, hsa-mir-99b, hsa-miR-99b-5p, hsa-mir-181a-2, hsa-mir-181a-1, hsa-miR-181a-5p, and combinations thereof.

[0011] In some embodiments said population of exosomes comprise a cytokine selected from the group consisting of the cytokines in Table 3 or Table 11, and combinations thereof.

[0012] In some embodiments said population of exosomes comprise a cytokine receptor in Table 4, and combinations thereof.

[0013] In some embodiments said population of exosomes comprise a protein selected from the group consisting of the proteins in Table 6, and combinations thereof.

[0014] In some embodiments said population of exosomes comprise a protein selected from the group consisting of Cytoplasmic aconitate hydratase, Cell surface glycoprotein MUC18, Protein arginine N-methyltransferase 1, Guanine nucleotide-binding protein G(s) subunit alpha, Cullin-5, Calcium-binding protein 39, Glucosidase 2 subunit beta, Chloride intracellular channel protein 5, Semaphorin-3B, 60S ribosomal protein L22, Spliceosome RNA helicase DDX39B, Transcriptional activator protein Pur-alpha, Programmed cell death protein 10, BRO1 domain-containing protein BROX, Kynurenine--oxoglutarate transaminase 3, Laminin subunit alpha-5, ATP-binding cassette sub-family E member 1, Syntaxin-binding protein 3, Proteasome subunit beta type-7, and combinations thereof.

[0015] In some embodiments said population of exosomes is a placental-derived population of exosomes. In some embodiments said placental-derived population of exosomes is derived from a media of a whole placenta culture. In some embodiments said placental-derived population of exosomes is derived from a media of a culture comprising placental lobes or portions of a

placenta. In some embodiments said placental-derived population of exosomes is derived from a media of a culture comprising placental stem cells, preferably placental-derived adherent cells (PDAC). In some embodiments the media is selected from the group consisting of a tissue culture media, a saline solution, and a buffered saline solution.

[0016] In some embodiments said population of exosomes comprise at least one marker molecule at a level at least two-fold higher than a population of exosomes derived from mesenchymal stem cells, cord blood, or placental perfusate. In some embodiments said population of exosomes comprise at least one marker molecule at a level at least two-fold lower than a population of exosomes derived from mesenchymal stem cells, cord blood, or placental perfusate.

[0017] The present invention also provides compositions comprising the populations of exosomes provided herein for use in the treatment of a disease, disorder, or condition in a subject.

[0018] The present invention also provides compositions comprising the populations of exosomes provided herein for use in the manufacture of a medicament for the treatment of a disease, disorder, or condition in a subject.

[0019] In some embodiments the disease, disorder or condition is a lung disease disorder or condition. In some embodiments the lung disease disorder or condition is selected from the group consisting of acute lung injury, acute and chronic diseases, asthma, chronic obstructive pulmonary disease (COPD), lung fibrosis, idiopathic pulmonary fibrosis, recovery of lung surgery after lung cancer, pulmonary embolism, acute respiratory distress syndrome, pneumonia, viral infection, coronavirus infection, Covid-19, and ventilator induced lung injury.

[0020] In some embodiments the disease, disorder or condition is a liver disease disorder or condition. In some embodiments the liver disease disorder or condition is selected from the group consisting of acute liver injury, acute and chronic diseases, liver cirrhosis, liver fibrosis, liver inflammation, metabolic disorders, liver damages caused by drugs, poisons, alcohol, virus (e.g., hepatitis) or other infectious disease, and cholestatic liver diseases.

[0021] In some embodiments the disease, disorder or condition is a brain / spinal cord disease disorder or condition. In some embodiments the brain / spinal cord disease disorder or condition is selected from the group consisting of acute brain / spinal cord injury, acute and chronic diseases, stroke, transient ischemic attach, Parkinson's and other movement disorders,

dementias, Alzheimer's diseases epilepsy / seizures, myelopathy, multiple sclerosis, infections of the central nervous system, spinal cord trauma, spinal cord inflammation, amyotrophic lateral sclerosis, spinal muscular atrophy.

[0022] In some embodiments the disease, disorder or condition is a kidney disease disorder or condition. In some embodiments the kidney disease disorder or condition is selected from the group consisting of acute kidney injury, acute and chronic diseases, kidney injury or damage induced by trauma, drugs (e.g., chemotherapeutic agents), kidney cysts, kidney stones, and kidney infections, recovery of kidney function after kidney transplant, diabetic nephropathy, and polycystic kidney disease.

[0023] In some embodiments the disease, disorder or condition is a gastrointestinal disease disorder or condition. In some embodiments the gastrointestinal disease disorder or condition is selected from the group consisting of acute gastrointestinal injury, autoimmune disease, acute and chronic diseases, Crohn's disease, irritable bowel syndrome, perianal abscesses, colitis, colon polyps and cancer.

[0024] In some embodiments the disease, disorder or condition is a bone marrow disease disorder or condition. In some embodiments the bone marrow disease disorder or condition is selected from the group consisting of acute and chronic diseases, anemia, leukopenia, thrombocytopenia aplastic anemia, myeloproliferative disorders, and stem cell transplantation.

[0025] In some embodiments the disease, disorder or condition is an eye disease disorder or condition. In some embodiments the eye disease disorder or condition is selected from the group consisting of acute eye injury, chronic and acute eye diseases, dry-eye syndrome and diabetic retinopathy, and macular degeneration.

[0026] In some embodiments the disease, disorder or condition is a spleen disease disorder or condition. In some embodiments the spleen disease disorder or condition is selected from the group consisting of acute spleen injury, chronic and acute spleen diseases, diseases associated with enlarged or de-regulated spleen functions, and lupus.

[0027] In some embodiments the disease, disorder or condition is a skin disease disorder or condition. In some embodiments the skin disease disorder or condition is selected from the group consisting of acute skin injury, chronic and acute skin diseases, diabetic foot ulcer, wound due to chemical burn, fire burn, skin or tissue damage caused, e.g., by injury, disease or surgical

procedures, hair loss, a hair follicle disease, disorder or condition, wrinkles, and reduced firmness.

[0028] In some embodiments the disease, disorder or condition is an ischemic disease disorder or condition. In some embodiments the ischemic disease disorder or condition is selected from the group consisting of acute ischemic injury, chronic and acute ischemic diseases, ischemic heart disease, ischemic vascular disease, ischemic colitis, mesenteric ischemia, Brain ischemia (e.g., stroke), acute or chronic limb ischemia, cutaneous ischemia, ischemic kidney, and the promotion of angiogenesis in tissues or organs in need thereof.

[0029] In some embodiments the disease, disorder or condition is a heart / cardiovascular disease disorder or condition. In some embodiments the heart / cardiovascular disease disorder or condition is selected from the group consisting of acute heart / cardiovascular injury, hypertension, atherosclerosis, myocardial infarction (MI), and chronic heart failure.

[0030] In some embodiments the disease, disorder or condition is an aging associated disease disorder or condition. In some embodiments the ageing associated disease disorder or condition is selected from the group consisting of age related fragility, age related diabetics, Alzheimer's diseases; age related macular degeneration, age related hearing loss, age related memory loss, age related cognitive decline, age related dementia, age related nuclear cataract, age associated loss of function and other effects of ageing.

[0031] In some embodiments the disease, disorder or condition is a systemic disease disorder or condition. In some embodiments the systemic disease disorder or condition is selected from the group consisting of acute and chronic diseases, graft versus host disease, and infections (e.g., ear infection).

[0032] In some embodiments the composition is formulated for intravenous administration. In some embodiments the composition is formulated for local injection. In some embodiments the composition is formulated for topical administration. In some embodiments the composition is formulated for inhalation. In some embodiments the composition is formulated for oral administration. In some embodiments the composition is formulated for subcutaneous administration. In some embodiments the composition is formulated for buccal or sublingual administration. In some embodiments the composition is formulated for administration to the ear. In some embodiments the composition is formulated for nasal administration. In some embodiments the composition is formulated for ocular administration.

[0033] In some embodiments the subject is a human.

[0034] In certain embodiments, purified exosomes are formulated into pharmaceutical compositions suitable for administration to a subject in need thereof. In certain embodiments, said subject is a human. The placenta-derived exosome-containing pharmaceutical compositions provided herein can be formulated to be administered locally, systemically subcutaneously, parenterally, intravenously, intramuscularly, topically, orally, intradermally, transdermally, or intranasally to a subject in need thereof. In a certain embodiment, the placenta-derived exosome-containing pharmaceutical compositions provided herein are formulated for local administration. In a certain embodiment, the placenta-derived exosome-containing pharmaceutical compositions provided herein are formulated for systemic subcutaneous administration. In a certain embodiment, the placenta-derived exosome-containing pharmaceutical compositions provided herein are formulated for parenteral administration. In a certain embodiment, the placenta-derived exosome-containing pharmaceutical compositions provided herein are formulated for intramuscular administration. In a certain embodiment, the placenta-derived exosome-containing pharmaceutical compositions provided herein are formulated for topical administration. In a certain embodiment, the placenta-derived exosome-containing pharmaceutical compositions provided herein are formulated for oral administration. In a certain embodiment, the placenta-derived exosome-containing pharmaceutical compositions provided herein are formulated for intradermal administration. In a certain embodiment, the placenta-derived exosome-containing pharmaceutical compositions provided herein are formulated for transdermal administration. In a certain embodiment, the placenta-derived exosome-containing pharmaceutical compositions provided herein are formulated for intranasal administration. In a specific embodiment, the placenta-derived exosome-containing pharmaceutical compositions provided herein are formulated for intravenous administration.

[0035] In another aspect, provided herein are uses of the exosomes and/or pharmaceutical compositions comprising exosomes described herein.

[0036] In a specific embodiment, the exosomes and/or pharmaceutical compositions comprising exosomes described herein are used to treat and/or prevent diseases and/or conditions in a subject in need thereof. In a specific embodiment, the exosomes and/or pharmaceutical compositions comprising exosomes described herein are used to promote angiogenesis and/or vascularization in a subject in need thereof. In another specific embodiment, the exosomes

and/or pharmaceutical compositions comprising exosomes described herein are used to modulate immune activity (*e.g.*, increase an immune response or decrease an immune response) in a subject in need thereof. In another specific embodiment, the exosomes and/or pharmaceutical compositions comprising exosomes described herein are used to repair tissue damage, *e.g.*, tissue damage caused by an acute or chronic injury, in a subject in need thereof.

[0037] In another specific embodiment, the derived exosomes and/or pharmaceutical compositions comprising exosomes described herein are for use in a method for treating and/or preventing diseases and/or conditions in a subject in need thereof. In another embodiment, the pharmaceutical compositions comprising exosomes described herein are for use in a method for treating diseases and/or conditions in a subject in need thereof. In another embodiment, the pharmaceutical compositions comprising exosomes described herein are for use in a method for preventing diseases and/or conditions in a subject in need thereof. In a specific embodiment, the pharmaceutical compositions comprising exosomes described herein are for use in a method for promoting angiogenesis and/or vascularization in a subject in need thereof. In another specific embodiment, the pharmaceutical compositions comprising exosomes described herein are for use in a method for modulating immune activity (*e.g.*, increase an immune response or decrease an immune response) in a subject in need thereof. In another specific embodiment, the pharmaceutical compositions comprising exosomes described herein are for use in a method for repairing tissue damage, *e.g.*, tissue damage caused by an acute or chronic injury, in a subject in need thereof.

[0038] In another specific embodiment, the exosomes and/or pharmaceutical compositions comprising exosomes described herein are used as cytoprotective agents. In another aspect, the exosomes and/or pharmaceutical compositions comprising exosomes described herein are provided in the form of a kit suitable for pharmaceutical use.

4. BRIEF DESCRIPTION OF THE DRAWINGS

[0039] **FIG. 1** shows a schematic for cultivating cells for exosome isolation.

[0040] **FIG. 2A – FIG.2C** show three pExo isolates that were analyzed for their size distribution by NanoSight. This work was performed and reported by SBI Inc. (System Bioscience Inc.) using a contract service (www.systembio.com/services/exosome-services/).

[0041] **FIG. 3A – FIG.3C** show protein markers present on pExo (N=12) (FIG. 3A) compared with placenta perfusate exosomes (FIG. 3B) and cord blood serum derived exosomes (FIG. 3C) using the MACSPlex Kit.

[0042] **FIG. 4** shows functional pathways of proteins identified in placental exosome populations.

[0043] **FIG. 5** shows common and unique protein identified in three placenta exosome samples.

[0044] **FIG. 6** shows that pExo promote migration of human dermal fibroblast cells in a transwell system.

[0045] **FIG. 7** shows that pExo promote migration of human umbicical cord vessel endothelial cells.

[0046] **FIG. 8** shows that pExo stimulate the proliferation of HUVEC.

[0047] **FIG. 9** shows that pExo stimulate the proliferation of human CD34+ cells.

[0048] **FIG. 10** shows that pExo stimulate the colony formation of human CD34+ cells.

[0049] **FIG. 11** shows that pExo inhibit the proliferation of SKOV3 cancer cells.

[0050] **FIG. 12** shows that pExo inhibit the proliferation of A549 cancer cells.

[0051] **FIG. 13** shows that pExo inhibit the proliferation of MDA321 cancer cells.

[0052] **FIG. 14** shows that pExo does not affect the proliferation of CD3+ T cells in culture.

[0053] **FIG. 15** shows that pExo increases expression of activation marker CD69 in UBC T CD3+ cells.

[0054] **FIG. 16** shows that pExo increases expression of activation marker CD69 in adult PBMC T CD3+ cells.

[0055] **FIG. 17** shows that pExo increases CD56+ NK cells in PBMC.

[0056] **FIG. 18** shows protein markers present on pExo (N=10) using MACSPlex Kit. Results show pExo are positive for the following protein markers including pExo are positive for CD2, CD4, CD8, CD14, CD24, CD29, CD31, CD40, CD42a, CD42b, CD44, CD45, CD49e, CD62P, CD63, CD69, CD81, CD86, CD105, CD133-1, CD142, CD146, CD326, HLA-ABC, HLA-DRDPDQ, MCSP, ROR1 and SSEA4.

[0057] **FIG. 19** shows that pExo stimulate proliferation of human kidney epithelial cells.

[0058] **FIG. 20** shows that pExo stimulate proliferation of human lung epithelial cells.

[0059] **FIG. 21** Top panel shows that pExo stimulate proliferation of human hepatic satellite cells. Bottom panel shows that pExo improves cell recovery comparing to media alone chemical-induced injury of liver cells by acetaminophen (APAP) (2 mM) or APAP + pExo(10 ug/ml) and acquire data with sweep interval every 15 minutes (N=3) in an xCELLigence Real Time Cell Analysis (RTCA).

[0060] **FIG. 22** Top panel shows that pExo stimulate proliferation of human dermal fibroblasts.

[0061] **FIG. 23** shows the study design of pExo biodistribution in vivo.

[0062] **FIG. 24** shows the in vivo bio-distribution of pExo (whole body imaging).

[0063] **FIG. 25** shows persistence of pExo in mice (whole body imaging).

[0064] **FIG. 26** shows bio-distribution of pExo in vivo (ex vivo imaging).

[0065] **FIG. 27** shows the study design of pExo effect on rat stroke model.

[0066] **FIG. 28** Top panel shows that pExo improved overall neuroscore significantly in rat after stroke induction. Bottom panels show that pExo-induced neurological deficit reduction compared to vehicle is superior than MSC-derived exosome in similar stroke models (left) and that pExo-induced neurological deficit reduction is superior than historic PDAC data in the same model (right).

[0067] **FIG. 29** shows that pExo improved body-swing significantly in rat after stroke induction.

[0068] **FIG. 30** shows that pExo improved forelimb placement score significantly in rats after stroke induction.

[0069] **FIG. 31** shows that pExo improved stepping test score significantly in rats after stroke induction.

[0070] **FIG. 32** shows pExo reduced lesion volume compared to vehicle control.

[0071] **FIG. 33** shows no lesion volume reduction by MSC-derived Exo was observed in a similar stroke model (Xin et al. 2013).

[0072] **FIG. 34** shows that pExo-induced lesion volume reduction is comparable to historic PDAC data in the same model.

[0073] **FIG. 35** shows that pExo significantly increased doublecortin positive cells in both subventricular zone (SVZ) and hippocampus suggesting enhanced neurogenesis.

[0074] **FIG. 36** shows that pExo significantly increased doublecortin positive cells in both subventricular zone (SVZ) and hippocampus suggesting enhanced neurogenesis.

[0075] **FIG. 37** shows the study design of the effect of pExo on mice with hindlimb ischemia (HLI).

[0076] **FIG. 38** shows that pExo improved the blood flow of mice with hindlimb ischemia (HLI) injury.

[0077] **FIG. 39** shows that pExo improved the blood flow of mice with hindlimb ischemia (HLI) injury.

[0078] **FIG. 40** shows the outline of an in vivo anti-aging study of pExo.

[0079] **FIG. 41** shows that the pExo-treated group had a longer latency to fall in rotarod test than vehicle group in the rotarod study.

[0080] **FIG. 42** shows that pExo-treated group had a quicker reduction of glucose at 30min after glucose administration than vehicle group as well as a lower glucose AUC.

[0081] **FIG. 43** shows the outline of an in vivo anti-GVHD study of pExo.

[0082] **FIG. 44** shows single or multiple dosing of pExo improved survival in GvHD model.

[0083] **FIG. 45** shows single or multiple dosing of pExo improved weight loss in GvHD model.

[0084] **FIG. 46** shows that multiple dosing of pExo inhibited the engraftment of CD3+ human T cells at Week 4 (mainly on CD4+ T cells).

[0085] **FIG. 47** shows that multiple dosing of pExo inhibited the engraftment of CD3+ human T cells at Week 4 (mainly on CD4+ T cells).

[0086] **FIG. 48** shows that pExo increases proliferation in PBTEC cells by multiple pExo cultivation methods.

[0087] **FIG. 49** shows that pExo increases proliferation in a dose dependent manner in PBTEC cells.

5. DETAILED DESCRIPTION

5.1. Placenta-Derived Exosomes

[0088] The placenta-derived exosomes described herein can be selected and identified by their morphology and/or molecular markers, as described below. The placenta-derived exosomes described herein are distinct from exosomes known in the art *e.g.*, chorionic villi mesenchymal

stem cell-derived exosomes, *e.g.*, those described in Salomon *et al.*, 2013, PLOS ONE, 8:7, e68451. Accordingly, the term "placenta-derived exosome," as used herein, is not meant to include exosomes obtained or derived from chorionic villi mesenchymal stem cells.

[0089] In certain embodiments, populations of placenta-derived exosomes described herein do not comprise cells, *e.g.*, nucleated cells, for example placental cells.

5.1.1. Placenta-Derived Exosome Markers

[0090] The placenta-derived exosomes described herein contain markers that can be used to identify and/or isolate said exosomes. These markers may, for example, be proteins, nucleic acids, saccharide molecules, glycosylated proteins, lipid molecules, and may exist in monomeric, oligomeric and/or multimeric form. In certain embodiments, the markers are produced by the cell from which the exosomes are derived. In certain embodiments, the marker is provided by the cell from which the exosomes are derived, but the marker is not expressed at a higher level by said cell. In a specific embodiment, the markers of exosomes described herein are higher in the exosomes as compared to the cell of origin when compared to a control marker molecule. In another specific embodiment, the markers of exosomes described herein are enriched in said exosomes as compared to exosomes obtained from another cell type (*e.g.*, the chorionic villi mesenchymal stem cells described in Salomon *et al.*, 2013, PLOS ONE, 8:7, e68451 and pre-adipocyte mesenchymal stem cells), wherein the exosomes are isolated through identical methods.

[0091] The three-dimensional structure of exosomes allows for the retention of markers on the surface of the exosome and/or contained within the exosome. Similarly, marker molecules may exist partially within the exosome, partially on the outer surface of the exosome and/or across the phospholipid bilayer of the exosome. In a specific embodiment, the markers associated with the exosomes described herein are proteins. In certain embodiments, the markers are transmembrane proteins that are anchored within the exosome phospholipid bilayer, or are anchored across the exosome phospholipid bilayer such that portions of the protein molecule are within the exosome while portions of the same molecule are exposed to the outer surface of the exosome. In certain embodiments, the markers are contained entirely within the exosome. In another specific embodiment, the markers associated with the exosomes described herein are nucleic acids. In certain embodiments, said nucleic acids are non-coding RNA molecules, *e.g.*, micro-RNAs (miRNAs).

5.1.1.1. Surface markers

[0092] The exosomes described herein comprise surface markers that allow for their identification and that can be used to isolate/obtain substantially pure populations of cell exosomes free from their cells of origin and other cellular and non-cellular material. Methods of for determining exosome surface marker composition are known in the art. For example, exosomal surface markers can be detected by fluorescence-activated cell sorting (FACS) or Western blotting.

[0093] In certain embodiments, the exosomes described herein comprise a surface marker at a greater amount than exosomes known in the art, as determinable by, e.g., FACS.

5.1.1.2. Yield

[0094] The exosomes described herein may be isolated in accordance with the methods described herein and their yields may be quantified. In a specific embodiment, the exosomes described herein are isolated at a concentration of about 0.5-5.0 mg per liter of culture medium (*e.g.*, culture medium with or without serum). In another specific embodiment, the exosomes described herein are isolated at a concentration of about 2-3 mg per liter of culture medium (*e.g.*, culture medium containing serum). In another specific embodiment, the exosomes described herein are isolated at a concentration of about 0.5-1.5 mg per liter of culture medium (*e.g.*, culture medium lacking serum).

5.1.2. Storage and Preservation

[0095] The exosomes described herein can be preserved, that is, placed under conditions that allow for long-term storage, or conditions that inhibit degradation of the exosomes.

[0096] In certain embodiments, the exosomes described herein can be stored after collection according to a method described above in a composition comprising a buffering agent at an appropriate temperature. In certain embodiments, the exosomes described herein are stored frozen, *e.g.*, at about -20°C or about -80°C.

[0097] In certain embodiments, the exosomes described herein can be cryopreserved, *e.g.*, in small containers, *e.g.*, ampoules (for example, 2 mL vials). In certain embodiments, the exosomes described herein are cryopreserved at a concentration of about 0.1 mg/mL to about 10 mg/mL.

[0098] In certain embodiments, the exosomes described herein are cryopreserved at a temperature from about -80°C to about -180°C. Cryopreserved exosomes can be transferred to

liquid nitrogen prior to thawing for use. In some embodiments, for example, once the ampoules have reached about -90°C, they are transferred to a liquid nitrogen storage area. Cryopreservation can also be done using a controlled-rate freezer. Cryopreserved exosomes can be thawed at a temperature of about 25°C to about 40°C before use.

[0099] In certain embodiments, the exosomes described herein are stored at temperatures of about 4°C to about 20°C for short periods of time (*e.g.*, less than two weeks).

5.2. Compositions

[00100] Further provided herein are compositions, *e.g.*, pharmaceutical compositions, comprising the exosomes provided herein. The compositions described herein are useful in the treatment of certain diseases and disorders in subjects (*e.g.*, human subjects) wherein treatment with exosomes is beneficial.

[00101] In certain embodiments, in addition to comprising the exosomes provided herein, the compositions (*e.g.*, pharmaceutical compositions) described herein comprise a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier," as used herein in the context of a pharmaceutically acceptable carrier, refers to a diluent, adjuvant, excipient, or vehicle with which the pharmaceutical composition is administered. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by JP Remington and AR Gennaro, 1990, 18th Edition.

[00102] In certain embodiments, the compositions described herein additionally comprise one or more buffers, *e.g.*, saline, phosphate buffered saline (PBS), Dulbecco's PBS (DPBS), and/or sucrose phosphate glutamate buffer. In other embodiments, the compositions described herein do not comprise buffers. In certain embodiments, the compositions described herein additionally comprise plasmalyte.

[00103] In certain embodiments, the compositions described herein additionally comprise one or more salts, *e.g.*, sodium chloride, calcium chloride, sodium phosphate, monosodium

glutamate, and aluminum salts (*e.g.*, aluminum hydroxide, aluminum phosphate, alum (potassium aluminum sulfate), or a mixture of such aluminum salts). In other embodiments, the compositions described herein do not comprise salts.

[00104] The compositions described herein can be included in a container, pack, or dispenser together with instructions for administration.

[00105] The compositions described herein can be stored before use, *e.g.*, the compositions can be stored frozen (*e.g.*, at about -20˚C or at about -80˚C); stored in refrigerated conditions (*e.g.*, at about 4˚C); or stored at room temperature.

5.2.1. Formulations and Routes of Administration

[00106] The amount of exosomes or a composition described herein which will be effective for a therapeutic use in the treatment and/or prevention of a disease or condition will depend on the nature of the disease, and can be determined by standard clinical techniques. The precise dosage of exosomes, or compositions thereof, to be administered to a subject will also depend on the route of administration and the seriousness of the disease or condition to be treated, and should be decided according to the judgment of the practitioner and each subject's circumstances. For example, effective dosages may vary depending upon means of administration, target site, physiological state of the patient (including age, body weight, and health), whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Treatment dosages are optimally titrated to optimize safety and efficacy.

[00107] Formulations of exosomes, e.g., of pExo, can be prepared for pharmaceutical or cosmetic uses in any convenient form such as a liquid, paste, or suspension. It can be formulated for administration by any necessary or convenient route of administration for a given indication including those suitable for parenteral (e.g., subcutaneous, intramuscular, intradermal, intravenous, or direct local injection), oral, inhalation (in solid and liquid forms or forms suitable for administration by a nebulizer), rectal, topical, buccal (e.g., sub-lingual), eyedrops, eardrops, cavity rinses (e.g., oral rinses) and transdermal administration.

[00108] Although the subject experiments were performed using placenta derived exosomes, applicants have demonstrated the effective delivery of intravenous exosome delivery to multiple organ systems. Accordingly, exosomes from other sources can be readily be delivered to these organ systems as taught and contemplated herein, for the treatment of the above conditions.

[00109] Administration of the exosomes described herein, or compositions thereof can be done via various routes known in the art. In certain embodiments, the exosomes described herein, or compositions thereof are administered by local, systemic, subcutaneous, parenteral, intravenous, intramuscular, topical, oral, intradermal, transdermal, or intranasal, administration. In a specific embodiment, said administration is via intravenous injection. In a specific embodiment, said administration is via subcutaneous injection. In a specific embodiment, said administration is topical. In another specific embodiment, the exosomes, or compositions thereof, are administered in a formulation comprising an extracellular matrix. In another specific embodiment, the exosomes, or compositions thereof, are administered in combination with one or more additional delivery device, *e.g.*, a stent. In another specific embodiment, the exosomes, or compositions thereof, are administered locally, *e.g.*, at or around the site of an area to be treated with said exosomes or compositions, such as hypoxic tissue (*e.g.*, in treatment of ischemic diseases) or draining lymph nodes.

5.3. Methods of Use

5.3.1. Treatment of Diseases that Benefit from Angiogenesis

[00110] The exosomes described herein, and compositions thereof, promote angiogenesis, and, therefore can be used to treat diseases and disorders that benefit from angiogenesis. Accordingly, provided herein are methods of using the exosomes described herein, or compositions thereof, to promote angiogenesis in a subject in need thereof. As used herein, the term "treat" encompasses the cure of, remediation of, improvement of, lessening of the severity of, or reduction in the time course of, a disease, disorder or condition, or any parameter or symptom thereof in a subject. In a specific embodiment, the subject treated in accordance with the methods provided herein is a mammal, *e.g.*, a human.

[00111] In one embodiment, provided herein are methods of inducing vascularization or angiogenesis in a subject, said methods comprising administering to the subject the exosomes provided herein, or a composition thereof. Accordingly, the methods provided herein can be used to treat diseases and disorders in a subject that that benefit from increased angiogenesis/vascularization. Examples of such diseases/conditions that benefit from increased angiogenesis, and therefore can be treated with the exosomes and compositions described herein included, without limitation, myocardial infarction, congestive heart failure, peripheral artery

disease, critical limb ischemia, peripheral vascular disease, hypoplastic left heart syndrome, diabetic foot ulcer, venous ulcer, or arterial ulcer.

[00112] In one embodiment, provided herein are methods of treating a subject having a disruption of blood flow, *e.g.*, in the peripheral vasculature, said methods comprising administering to the subject the exosomes provided herein, or a composition thereof. In a specific embodiment, the methods provided herein comprise treating a subject having ischemia with the exosomes provided herein, or a composition thereof. In certain embodiments, the ischemia is peripheral arterial disease (PAD), *e.g.*, is critical limb ischemia (CLI). In certain other embodiments, the ischemia is peripheral vascular disease (PVD), peripheral arterial disease, ischemic vascular disease, ischemic heart disease, or ischemic renal disease.

5.3.2. Patient Populations

[00113] In certain embodiments, the exosomes described herein are administered to a subject in need of therapy for any of the diseases or conditions described herein. In another embodiment, a composition described herein is administered to a subject in need of therapy for any of the diseases or conditions described herein. In certain embodiments said subject is a human.

[00114] In a specific embodiment, the exosomes or compositions described herein are administered to a subject (*e.g.*, a human) in need of a therapy to increase angiogenesis and/or vascularization.

5.4. Kits

[00115] Provided herein is a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions described herein, *i.e.*, compositions comprising the exosomes described herein. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[00116] The kits described herein can be used in the above methods. The compositions described herein can be prepared in a form that is easily administrable to an individual. For example, the composition can be contained within a container that is suitable for medical use. Such a container can be, for example, a sterile plastic bag, flask, jar, or other container from which the compositions can be easily dispensed. For example, the container can be a blood bag

or other plastic, medically acceptable bag suitable for the intravenous administration of a liquid to a recipient.

Exemplary Placenta Culture

[00117] The placenta is a reservoir of cells, including stem cells such as hematopoietic stem cells (HSC) and non-hematopoietic stem cells. Described herein are methods to isolate exosomes from a placenta or portion thereof, which is cultured in a bioreactor. Exosomes are secreted by the cells during the culture and the exosomes are secreted into the media, which facilitates further processing and isolation of the exosomes. Exosomes can be also isolated from the placenta or portion thereof at different stages of culture (e.g., at different time points and different perfusion liquids may be used at each recovery step). Once in the media, the exosomes can be further isolated using e.g., centrifugation, a commercially available exosome isolation kit, lectin affinity, and/or affinity chromatography (e.g., utilizing immobilized binding agents, such as binding agents attached to a substrate, which are specific for a small Rab family GTPase, annexin, flotillin, Alix, Tsg101, ESCRT complex, CD9, CD37, CD53, CD63, CD63A, CD81, CD82), Hsp70, Hsp90, epithelial cell adhesion molecules (EpCam), perforin, TRAIL, granzyme B, Fas, one or more cancer markers such as: Fas ligand, CD24, EpCAM, EDIL3, fibronectin, Survivin, PCA3, TMPRSS2:ERG, Glypican-1, TGF-β1, MAGE 3/6, EGFR, EGFRvIII, CD9, CD147, CA-125, EpCam, and/or CD24, or one or more inflammatory or pathogenic markers such as: a viral, fungal, or a bacterial protein or peptide including but not limited to α-synuclein, HIV or HCV proteins, tau, beta-amyloid, TGF-beta, TNF-alpha, fetuin-A, and/or CD133) . The isolated exosomes can be used for therapeutics, diagnostics, and as biotechnological tools.

[00118] "Exosomes" as described herein are vesicles that are present in many and perhaps all eukaryotic fluids, including ascites fluid, blood, urine, serum and breast milk. They may also be referred to as extracellular vesicles. Exosomes are bi-lipid membrane vesicles secreted from living cells that play important functions in cell-cell communications. Exosomes are produced by cells, such a stem cells, epithelial cells and a sub-type of exosomes, defined as Matrix-bound nanovesicles (MBVs), was reported to be present in extracellular matrix (ECM) bioscaffolds (non-fluid). The reported diameter of exosomes is between 30 and 100 nm, which is larger than low-density lipoproteins (LDL) but much smaller than, for example, red blood cells. Exosomes can be released from the cell when multivesicular bodies fuse with the plasma membrane or released directly from the plasma membrane.

[00119] Exosomes have been shown to have specialized functions and play a key role in processes such as coagulation, intercellular signaling, and waste management. It is known that extracellular vesicles and exosomes secreted by placenta contribute to the communication between placenta and maternal tissues to maintain maternal-fetal tolerance. Exosomes isolated from human placental explants was shown to have immune modulation activities. Stem cell derived exosomes were also shown to reduce neuroinflammation by suppressing the activation of astrocytes and microglia and promote neurogenesis possibly by targeting the neurogenic niche, both which contribute to nervous tissue repair and functional recovery after TBI. (Review Yang et al. 2017, Frontiers in Cellular Neuroscience). Exosomes derived from human embryonic mesenchymal stem cells also promote osteochondral regeneration (Zhang et al. 2016, Osteoarthritis and Cartilage). Exosomes secreted by human placenta that carry functional Fas Ligand and Trail molecules were shown to convey apoptosis in activated immune cells, suggesting exosome-mediated immune privilege of the fetus. (Ann-Christin Stenqvist et al., Journal of Immunology, 2013, 191: doi:10.4049).

[00120] Exosomes contain active biologics including lipids, cytokines, microRNA, mRNA and DNA. They may also function as mediators of intercellular communication via genetic material and/or protein transfer. Exosomes may also contain cell-type specific information that may reflect a cell's functional or physiological state. Consequently, there is a growing interest in the development of clinical and biological applications for exosomes.

[00121] Accordingly, exosomes isolated from human placenta or a portion thereof using the approaches described herein, optionally including characterization of said exosomes (e.g., by identifying the presence or absence of one or more proteins or markers on the exosomes) can be used to stimulate an immuno-modulation, an anti-fibrotic environment, and/or a pro-regenerative effect. Accordingly, exosomes isolated from human placenta or a portion thereof using the approaches described herein may be selected (e.g., according to markers present or absent on the exosomes), purified, frozen, lyophilized, packaged and/or distributed as a therapeutic product and/or a biotechnological tool.

[00122] In some alternatives, it may be beneficial to identify exosomes having tumor markers or peptides, pathogenic markers or peptides, such as viral, fungal, or bacterial markers or peptides, and/or inflammatory markers, such as inflammatory peptides, so that such exosomes can be removed from a population of exosomes (e.g., removal by affinity chromatography with

binding molecules such as, antibodies or binding portions thereof, which are specific for such tumor markers or peptides, pathogenic markers or peptides, and/or inflammatory markers or peptides). Accordingly, in some alternatives, for example, a first population of exosomes are isolated from human placenta or a portion thereof by the methods described herein and once the first population of exosomes is isolated this population of exosomes is further processed to remove one or more subpopulations of exosomes using a substrate having an immobilized antibody or binding portion thereof (e.g., a membrane, a resin, a bead, or a vessel having said immobilized antibody or binding portion thereof), wherein the immobilized antibody or binding portion thereof is specific for a marker or peptide present on the subpopulation of exosomes, which are selected for further isolation, such as, one or more tumor markers or peptides, pathogenic markers or peptides, e.g., viral, fungal, or bacterial markers or peptides, and/or inflammatory markers or inflammatory peptides. In some alternatives, a first population of exosomes isolated from human placenta or a portion thereof by the methods described herein are contacted with a substrate having an immobilized antibody or binding portion thereof (e.g., a membrane, a resin, a bead, or a vessel having said immobilized antibody or binding portion thereof), wherein the immobilized antibody or binding portion thereof is specific for one or more cancer markers such as: Fas ligand, CD24, EpCAM, EDIL3, fibronectin, Survivin, PCA3, TMPRSS2:ERG, Glypican-1, TGF-β1, MAGE 3/6, EGFR, EGFRvIII, CD9, CD147, CA-125, EpCam, and/or CD24 so as to isolate a second population of exosomes from the first population of exosomes based on the affinity to the immobilized antibody or binding portion thereof. In some alternatives, a first population of exosomes isolated from human placenta or a portion thereof by the methods described herein are contacted with a substrate having an immobilized antibody or binding portion thereof (e.g., a membrane, a resin, a bead, or a vessel having said immobilized antibody or binding portion thereof), wherein the immobilized antibody or binding portion thereof is specific for one or more inflammatory or pathogenic markers such as: a viral, fungal, or a bacterial protein or peptide including but not limited to α-synuclein, HIV or HCV proteins, tau, beta-amyloid, TGF-beta, TNF-alpha, fetuin-A, and/or CD133 or portions thereof so as to isolate a second population of exosomes from the first population of exosomes based on the affinity to the immobilized antibody or binding portion thereof.

[00123] In some alternatives, the population of exosomes isolated and/or selected by the approaches described herein have markers or peptides that are useful for therapeutics such as

perforin and/or granzyme B, which has been shown to mediate anti-tumor activity both *in vitro* and *in vivo* (*J Cancer* 2016; 7(9):1081-1087) or Fas, which has been found in exosomes that exert cytotoxic activity against target cancer cells. (*Theranostics* 2017; 7(10):2732-2745). Accordingly, in some alternatives, a first population of exosomes isolated from human placenta or a portion thereof by the methods described herein are contacted with a substrate having an immobilized antibody or binding portion thereof (e.g., a membrane, a resin, a bead, or a vessel having said immobilized antibody or binding portion thereof), wherein the immobilized antibody or binding portion thereof is specific for perforin, TRAIL and/or granzyme B and/or Fas and a second population of exosomes from the first population of exosomes is isolated based on the affinity to the immobilized antibody or binding portion thereof to perforin, TRAIL and/or granzyme B and/or Fas. In some alternatives, a population of exosomes is isolated, which comprises CD63 RNAs, and/or a desired microRNA. In some alternatives, a population of exosomes is isolated and/or characterized after isolation using affinity chromatography or immunological techniques, wherein said population of exosomes comprise markers or peptides such as small Rab family GTPases, annexins, flotillin, Alix, Tsg101, ESCRT complex, CD9, CD37, CD53, CD63, CD63A, CD81, CD82), Hsp70, Hsp90) and/or epithelial cell adhesion molecules (EpCam). As detailed above, in some alternatives, a first population of exosomes isolated from human placenta or a portion thereof by the methods described herein are contacted with a substrate having an immobilized antibody or binding portion thereof (e.g., a membrane, a resin, a bead, or a vessel having said immobilized antibody or binding portion thereof), wherein the immobilized antibody or binding portion thereof is specific for small Rab family GTPases, annexins, flotillin, Alix, Tsg101, ESCRT complex, CD9, CD37, CD53, CD63, CD63A, CD81, CD82), Hsp70, Hsp90) and/or epithelial cell adhesion molecules (EpCam) and a second population of exosomes from the first population of exosomes is isolated based on the affinity to the immobilized antibody or binding portion thereof to small Rab family GTPases, annexins, flotillin, Alix, Tsg101, ESCRT complex, CD9, CD37, CD53, CD63, CD63A, CD81, CD82), Hsp70, Hsp90) and/or epithelial cell adhesion molecules (EpCam). In other alternatives, a population of exosomes isolated from human placenta or a portion thereof by the methods described herein are contacted with an antibody or binding portion thereof specific for one or more of small Rab family GTPases, annexins, flotillin, Alix, Tsg101, ESCRT complex, CD9, CD37, CD53, CD63, CD63A, CD81, CD82, Hsp70, Hsp90 and/or epithelial cell adhesion

molecules (EpCam) and the binding of the antibody or binding portion thereof is detected with a secondary binding agent having a detectable reagent, which binds to said antibody or binding portion thereof (e.g., utilizing an ELISA or blotting procedure) so as to confirm the presence of the small Rab family GTPases, annexins, flotillin, Alix, Tsg101, ESCRT complex, CD9, CD37, CD53, CD63, CD63A, CD81, CD82), Hsp70, Hsp90 and/or epithelial cell adhesion molecules (EpCam) in the isolated exosome population.

[00124] "Isolation" as described herein is a method for separating the exosomes from other materials. Isolation of exosomes may be performed by high centrifugal force in a centrifuge, utilization of commercially available kits (e.g. SeraMir Exosome RNA Purification kit (SBI system biosciences), Intact Exosome Purification and RNA Isolation (CombinationKit) Norgen BioTek Corp.), and the use of lectin affinity or affinity chromatography with binding agents (e.g., an antibody or binding portion thereof) specific for markers or peptides on the exosomes such as the markers or peptides mentioned above (e.g., binding agents specific for small Rab family GTPases, annexins, flotillin, Alix, Tsg101, ESCRT complex, CD9, CD37, CD53, CD63, CD63A, CD81, CD82), Hsp70, Hsp90, epithelial cell adhesion molecules (EpCam), perforin, TRAIL, granzyme B, Fas, one or more cancer markers such as: Fas ligand, CD24, EpCAM, EDIL3, fibronectin, Survivin, PCA3, TMPRSS2:ERG, Glypican-1, TGF-β1, MAGE 3/6, EGFR, EGFRvIII, CD9, CD147, CA-125, EpCam, and/or CD24, or one or more inflammatory or pathogenic markers such as: a viral, fungal, or a bacterial protein or peptide including but not limited to α-synuclein, HIV or HCV proteins, tau, beta-amyloid, TGF-beta, TNF-alpha, fetuin-A, and/or CD133).

[00125] "Placenta" as described herein is an organ in the uterus of pregnant eutherian mammals, nourishing and maintaining the fetus through the umbilical cord. As described herein, the placenta may be used as a bioreactor for obtaining exosomes. In some alternatives, a decellularized placenta may be used as a scaffold and bioreactor, which harbors an exogenous cell population (e.g., a cell population that has been seeded onto and cultured with the decellularized placenta) so as to obtain a population of exosomes from said cells, which are cell specific. Accordingly, in some alternatives, decellularized placenta is seeded with a regenerative cell population (e.g., a population of cells comprising stem cells and/or endothelial cells and/or progenitor cells) and said regenerative cell population is cultured on said decellularized placenta in a bioreactor and cell specific exosomes are isolated from said cultured cells using

centrifugation, a commercially available exosome isolation kit, lectin affinity, and/or affinity chromatography using a binding agents (e.g., an antibody or binding portion thereof) specific for markers or peptides on the exosomes such as the markers or peptides mentioned above (e.g., binding agents specific for small Rab family GTPases, annexins, flotillin, Alix, Tsg101, ESCRT complex, CD9, CD37, CD53, CD63, CD63A, CD81, CD82), Hsp70, Hsp90, epithelial cell adhesion molecules (EpCam), perforin, TRAIL, granzyme B, Fas, one or more cancer markers such as: Fas ligand, CD24, EpCAM, EDIL3, fibronectin, Survivin, PCA3, TMPRSS2:ERG, Glypican-1, TGF-β1, MAGE 3/6, EGFR, EGFRvIII, CD9, CD147, CA-125, EpCam, and/or CD24, or one or more inflammatory or pathogenic markers such as: a viral, fungal, or a bacterial protein or peptide including but not limited to α-synuclein, HIV or HCV proteins, tau, beta-amyloid, TGF-beta, TNF-alpha, fetuin-A, and/or CD133).

[00126] "Ascites fluid" as described herein is excess fluid in the space between the membranes lining the abdomen and abdominal organs (the peritoneal cavity). Ascites fluid may be a source of exosomes.

[00127] "Plasma" as described herein is the liquid part of the blood and lymphatic fluid, which makes up about half of the volume of blood. Plasma is devoid of cells and, unlike serum, has not clotted. Blood plasma contains antibodies and other proteins. Plasma may be a source of exosomes.

[00128] Several methods of culturing cells so as to produce copious amounts exosomes are provided herein. Culture media used for recovering or isolating the exosomes may be provided with one or more nutrients, enzymes or chelators. Chelators may be used to facilitate release of the exosomes from the cultured cells. Without being limiting, chelators used in some of the methods may include a phosphonate, BAPTA tetrasodium salt, BAPTA/AM, Di-Notrophen TM reagent tetrasodium salt, EGTA/AM, pyridoxal isonicotinoyl hydrazine, N,N,N′,N′-tetrakis-(2 Pyridylmethyl)ethylenediamine, 6-Bromo-N′-(2-hydroxybenzylidene)-2-methylquinoline-4-carbohydrazide, 1,2-Bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrakis(acetoxymethyl ester), (Ethylenedinitrilo)tetraacetic acid, (EDTA), Edathamil, Ethylenedinitrilotetraacetic acid, Ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid, or Ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) or any combination thereof. The chelator may be provided in the media used to culture or isolate the exosomes at a concentration of 1 mM, 2mM, 3mM, 4mM, 5mM, 6mM, 7mM, 8mM, 9mM,10

mM, 20mM, 30mM, 40mM, 50mM, 60mM, 70mM, 80mM, 90mM or 100mM or at a concentration that is within a range defined by any two aforementioned concentrations. As shown herein, the presence of one or more chelators in the media unexpectedly enhanced recovery of exosomes from placenta cultured in a bioreactor. The media used to culture and/or recover the exosomes may also have a protease, which may further enhance the release of exosomes. In some alternatives, the protease provided in the media is trypsin, collagenase, chymotrypsin or carboxypeptidase. In some alternatives, the protease is provided in the media at a concentration of 1 mM, 2mM, 3mM, 4mM, 5mM, 6mM, 7mM, 8mM, 9mM, 10 mM, 20mM, 30mM, 40mM, 50mM, 60mM, 70mM, 80mM, 90mM or 100mM or at a concentration that is within a range defined by any two of the aforementioned concentrations. One or more sugars may also be added to the media used to culture and/or recover the exosomes. In some alternatives, the sugar added to the media is glucose. It is contemplated that the presence of glucose in the media enhances the release of the exosomes. In some alternatives, the glucose is provided in the media at a concentration of 1 mM, 2mM, 3mM, 4mM, 5mM, 6mM, 7mM, 8mM, 9mM, 10 mM, 20mM, 30mM, 40mM, 50mM, 60mM, 70mM, 80mM, 90mM or 100mM or at a concentration that is within a range defined by any two of the aforementioned concentrations. The media may also include growth factors, cytokines, or one or more drugs e.g., GM-CSF, serum and/or an AHR antagonist.

Methods of collecting exosomes from a placenta or portion thereof

[00129] An exemplary method for recovery of exosomes from placenta is shown in **Figure 1.** Sources for the exosome isolation may be from cord blood plasma: PRP, placenta perfusate (PS), placenta tissue cultivate (PTS), placenta organ cultivate (PO), or exogenous cells that may be placed in the placenta or portion thereof, when the placenta is used as a bioreactor for exosome generation. By one approach, placenta or portion thereof is collected (#200010323, collected 9/25/2017). Placenta is contacted with a media or perfused with normal PSC-100 collection methods, collected as PS-1 (9/26/2017). The placenta or portion thereof is incubated in a hood for at least 4 hours. The placenta or portion thereof is contacted with media (RPMI media) or perfused with 500mL RPMI base medium (1% antibiotics), collected as PS-2. The placenta or portion thereof is then incubated in a hood overnight and is covered. The placenta or portion thereof is contacted with or perfused with 750mL saline solution and collected as PS-3. The

samples were then shipped to a laboratory for analysis (Warren). PS1, PS2 and PS3 were analyzed by FACS at the same day after RBC lysis.

[00130] For the analysis, placenta tissue were cut into 1x1x1 cm size, placed in 100 mL of solution (all with 1% P&S) in T75 flasks (each about 1/8 of the placenta). Four solutions were assayed: A: DMEM medium; B: PBS; C: PBS+5mM EDTA; D: PBS+0.025% Trypsin-EDTA. This was then allowed to incubate in 37°C incubator overnight (O/N).

[00131] The supernatant was then harvested, passed through tissue filter and spun down at 400g to harvest cells (pellet). The supernatant after the first centrifugation was then spun down for exosome isolation (3000g spin soup>10,000 spin soup: 100,000g pellet)

[00132] The cells collected were also used for FACS analysis. The cell samples were in several buffers (A=PTS1; B=PTS2; C=PTS-3, D=PTS4). Exosomes were recovered and were then assayed to identify the presence of an exosome marker confirming that the exosomes were obtained and isolated by the procedure.

Identification of a population of exosomes isolated from the placental bioreactor using ELISA and protein assays

[00133] Fractions of supernatant from the placental bioreactor were collected by the methods described above and the fractions were filtered. The supernatant was then subjected to centrifugation at 400g x 10 min to collect the cells. After the first centrifugation, a second centrifugation was performed at 3000g x 30 min to pellet cell debris. A third centrifugation was the performed at 10,000g x 1 hr to pellet micro vesicles. A fourth centrifugation was then performed at 100,000g x 1.5 hr to pellet exosomes. The centrifuge tube containing the pelleted exosomes was then placed upside-down on paper to drain residual liquid. The exosome pellet was then dissolved in an appropriate volume of sterile PBS (e.g. 2.0 mL) to dissolve pellet, and the solution containing the exosomes was then aliquoted in a sterile Eppendorf tube and frozen in a -20°C/-80°C freezer. Exosomes were then assayed for the presence of an exosome-specific marker CD63A using an ELISA-63A and Protein Quantification Kit.

As shown, PRP, placenta perfusate and placenta tissue contain a population of exosomes that are CD63+ and can be efficiently isolated by ultracentrifiguation. For the exosome isolation, first the culture supernatant was filtered through a tissue filter and several centrifugations were performed as described above to obtain the exosomes, which were then frozen. For the ELISA detection of

the exosomes, an anti-CD63 antibody was used. The sample was diluted 1:1 with exosome binding buffer (60uL + 60uL) in the assay. CD63+ exosomes were efficiently isolated by this procedure.

Characterization of exosomes

[00134] Exosomes may contain protein, peptides, RNA, DNA and cytokines. Methods such as miRNA sequencing, surface protein analysis (MACSPlex Exosome Kit, Miltenyi), proteomic analysis, functional studies (enzyme assays in vitro wound healing assays (scratch assay), exosome-induced cell proliferation (human keratinocytes or fibroblast) (comparing to 5 known stimulants), exosome-induced collagen production (human keratinocyte or fibroblast): comparing to TGFb, includes serum and non-serum control, ELISA for pro-collagen 1 C peptide, exosome-induced inhibition of inflammatory cytokines: response cell types include human keratinocytes or human fibroblasts, and comparisons to lyophilized heat-killed bacterial or LPS) may be performed.

[00135] In some alternatives, isolated exosomes were concentrated with 100-Kda Vivaspin filter (Sartorius), washed once with PBS and approximately 40uL was recovered. The concentrated population of exosomes was mixed with 10uL of 5XRIPA lysis buffer containing 1xprotease inhibitor cocktail (Roche) and vortexed, which was then followed by sonication at 20°C for 5 min at a water sonicator (Ultrasonic Cleaner, JSP). After sonication, the tube was incubated on ice for 20 min with intermittent mixing. Next, the mixture was centrifuged at 10,000g for 10 min at 4°C. The isolated clear lysate was transferred to a fresh tube. The protein amount was measured with BCA kit and 10ug of protein was loaded per lane for Western blotting and an antibody is used for determination of a protein of interest.

[00136] In another alternative, exosome labeling and uptake by cells is examined (e.g. HEK293T). An aliquot of frozen eluted exosomes were resuspended in 1 mL of PBS and labeled using PKH26 Fluorescent cell linker Kits (Sigma-Aldrich). A 2x PNK26-dye solution (4uL dye in 1 mL of Diluent C) was prepared and mixed with 1 mL of exosomal solution for a final dye concentration of 2x10e-6M. The samples was immediately mixed for 5 min and staining was stopped by adding 1% BSA to capture excel PKH26 dye. The labeled exosomes was transferred into a 100-Kda Vivaspin filter and spun at 4000g then washed with PBS twice and approximately 50uL of sample was recovered for analysis of exosome concentration using NTA prior to storage at -80C. PBS was used as negative control for the labeling reaction. To perform the uptake studies, HEK293T cells were plated in 8-well chamber slide (1x10e4/well) using

regular medium. After 24hr, the slides was washed twice with PBS and incubated with DMEM-exo-free FBS (10%) for 24hr. Following this, fresh DMEM media with 10% exo-free PBS (200uL) each labeled exosome sample, corresponding to 2x10e9 exosomes, was added to each well and incubated for 1.5 hr in a cell culture incubator. After incubation, the slides was washed twice with PBS (500ul) and fixed with 4% paraformaldehyde solution for 20 min at room temperature. The slides were washed twice with PBS (500uL) , dried, and mounted using a ProLong Gold Antifade Reagent with DAPI. The cells were visualized using an Axioskop microscope (Zeiss)

High yield isolation of exosomes from cultivated postpartum human placenta

[00137] Postpartum human placentas obtained with full donor consent were perfused. Residual blood from the placenta was washed off with a large volume of sterile saline and then cultivated in a 5-L bioreactor with serum free culture medium supplemented with antibiotics and cultivated at 37°C incubator (5% CO2) and alternated with rotating at refrigerated conditions for extended period unto to 4 days. Supernatant of the culture medium was processed by sequential centrifugation by 3000g and 10,000g to pellet tissue, cell and micro-vesicles. Exosomes were pelleted by 100,000g ultra-centrifugation from the supernatant of 10,000g centrifugation and dissolved with sterile PBS. The yield of exosome was quantified by BCA protein assay.

[00138] Supernatants from the placenta organ culture were processed as described in the methods to isolate exosomes. An ELISA assay using anti-CD63A antibodies demonstrated that the isolated exosomes contain the CD63A protein, a specific protein marker for exosomes. It is estimated one placenta cultured in one liter of medium generated approximately 40mg of exosomes, or approximately $1x10^{13}$ CD63A positive exosome particles in 24 hours. Further characterization of these placenta-organ derived exosomes including expression of CD9, CD81, size and functional activities are performed.

[00139] In another set of experiments, postpartum human placentas obtained with full donor consent are perfused to isolate exosomes with media's having different concentrations of EDTA. Serum free culture medium supplemented with antibiotics and varying concentrations of EDTA (e.g., 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100mM or within a range defined by any two of the aforementioned concentrations) are perfused into placenta through umbilical cord veins via peristaltic pump with a constant rate and cultivated another 24-48 hours under controlled conditions. Following this cultivation, 750mL of physiologic medium containing the amount of

EDTA employed is perfused at controlled rate. Exosomes are then isolated by sequential centrifugation and ultracentrifugation, confirmed by the CD63A ELISA assay, and quantified by the BCA protein assay, all described above. It will be shown that the concentration of EDTA in the media used to recover the exosomes impacts the amount of exosomes recovered from the placenta cultured in the bioreactor.

Additional alternatives

[00140] In some alternatives, a method of exosome isolation from a placenta or a portion thereof is provided. The method comprises a) contacting the placenta or a portion thereof with a first medium; b) obtaining a first fraction comprising exosomes from said placenta or portion thereof; c) contacting said placenta or portion thereof with a second medium; d) obtaining a second fraction comprising exosomes from said placenta or portion thereof; e) contacting said placenta or portion thereof with a third medium; f) obtaining a third fraction comprising exosomes from said placenta or portion thereof and, optionally, isolating the exosomes from said first, second, and/or third fractions. In some alternatives, the method further comprises multiple steps of contacting the placenta or portion thereof with an additional medium; and obtaining an additional fraction comprising exosomes from said placenta or portion thereof. These two steps may be repeated multiple times. Preferably, the placenta or portion thereof is cultured and/or maintained in a bioreactor. In some alternatives, the placenta or portion thereof comprises amniotic membrane. In some alternatives, the placenta or a portion thereof is a human placenta or a portion thereof. In some alternatives, the first, second, and/or third mediums are in contact with the placenta or portion thereof for at least 45 minutes, such as 45 minutes or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 hours or any amount of time that is within a range defined by any two of the aforementioned time points. In some alternatives, the first, second, and/or third mediums are in contact with the placenta or portion thereof for at least 7, 14, 28, 35 or 42 days or any amount of time that is within a range defined by any two of the aforementioned time points. In some alternatives, the placenta or a portion thereof has been minced, ground, or treated with an enzyme such as collagenase and/or a protease.

[00141] In some alternatives, a placenta or a portion thereof is provided as a substantially flat or sheet-like scaffold material, which has been decellularized and, optionally, substantially dried. The decellularized placenta or a portion thereof is used as a scaffold to harbor exogenous cells

such as homogeneous cell populations obtained from cell culture or primary isolation procedures (e.g., regenerative cells including stem cells, endothelial cells, and/or progenitor cells). The method further comprises passaging fluid or fluid comprising the cells to be seeded into the decellularized placenta or portion thereof. Once the cells are established, exosomes generated from the cells are recovered and isolated using the procedures described above. In some alternatives, the fluid comprising the cells to be seeded on the decellularized placenta or portion thereof is ascites fluid, blood or plasma. In some alternatives, the cells are from an organ. In some alternatives, the cells are from liver, kidney, lung or pancreas. In some alternatives, the cells are immune cells. In some alternatives, the cells are T-cells or B-cells.

[00142] In some alternatives, the first medium comprises Phosphate buffered saline (PBS). In some alternatives, the second medium comprises growth factors. In some alternatives, the third medium comprises a chelator. In some alternatives, the chelator is EDTA, EGTA, a phosphonate, BAPTA tetrasodium salt, BAPTA/AM, Di-Notrophen TM reagent tetrasodium salt, EGTA/AM, pyridoxal isonicotinoyl hydrazine, N,N,N′,N′-tetrakis-(2 Pyridylmethyl)ethylenediamine, 6-Bromo-N′-(2-hydroxybenzylidene)-2-methylquinoline-4-carbohydrazide, 1,2-Bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrakis(acetoxymethyl ester), (Ethylenedinitrilo)tetraacetic acid, EDTA, Edathamil, Ethylenedinitrilotetraacetic acid, Ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid, or Ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid tetrasodium salt or any combination thereof. In some alternatives, the chelator is EDTA or EGTA or a combination thereof. In some alternatives, the chelator is provided in the third medium at a concentration of 1 mM, 2mM, 3mM, 4mM, 5mM, 6mM, 7mM, 8mM, 9mM, 10mM, 20mM, 30mM, 40mM, 50mM, 60mM, 70mM, 80mM, 90mM or 100mM or at a concentration that is within a range defined by any two aforementioned concentrations. In some alternatives, the concentration of EDTA in the third medium is provided at a concentration of 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM 10 mM, 20 mM, 30 mM, 40 mM, 50 mM, 60 mM, 70 mM, 80 mM, 90 mM or 100 mM or at a concentration that is within a range defined by any two aforementioned concentrations.

[00143] In some alternatives, the third medium comprises a protease. In some alternatives, the protease is a trypsin, collagenase, chymotrypsin or carboxypeptidase or a mixture thereof. In some alternatives, the protease is trypsin. In some alternatives, the protease is provided in the third medium at a concentration of 1 mM, 2mM, 3mM, 4mM, 5mM, 6mM, 7mM, 8mM, 9mM,

10 mM, 20mM, 30mM, 40mM, 50mM, 60mM, 70mM, 80mM, 90mM or 100mM or at a concentration that is within a range defined by any two of the aforementioned concentrations.

[00144] In some alternatives, the method further comprises contacting the placenta or portion thereof with an additional plurality of mediums, wherein the contacting results in obtaining multiple fractions comprising exosomes. In some alternatives, the first, second, third or additional mediums comprise glucose. In some alternatives, the first, second, third or additional mediums comprise GM-CSF. In some alternatives, the first, second, third or additional mediums comprise serum. In some alternatives, the first, second, third or additional mediums comprise DMEM. In some alternatives, the first, second, third or additional medium comprises an AHR antagonist. In some alternatives, the AHR antagonist is SR1. In some alternatives, the SR1 is at a concentration of 1nM, 10nM, 100nM, 200nM, 300nM, 400nM, 500nM, 600nM, 700nM, 800nM, 900nM or 1mM or any other concentration within a range defined by any two aforementioned values.

[00145] In some alternatives, the first medium is in contact with the placenta or portion thereof while maintaining a temperature of 0 ºC, 5 ºC, 10 ºC, 15 ºC, 20 ºC, 25 ºC, 30 ºC, 35 ºC or 40 ºC or a temperature that is within a range defined by any two of the aforementioned temperatures. In some alternatives, the second medium is in contact with the placenta or portion thereof while maintaining a temperature of 0 ºC, 5 ºC, 10 ºC, 15 ºC, 20 ºC, 25 ºC, 30 ºC, 35 ºC or 40 ºC or a temperature that is within a range defined by any two of the aforementioned temperatures. In some alternatives, the third medium is in contact with the placenta or portion thereof while maintaining a temperature of 0 ºC, 5 ºC, 10 ºC, 15 ºC, 20 ºC, 25 ºC, 30 ºC, 35 ºC or 40 ºC or a temperature that is within a range defined by any two of the aforementioned values. In some alternatives, the additional plurality of mediums is in contact with the placenta or portion thereof while maintaining a temperature of 0 ºC, 5 ºC, 10 ºC, 15 ºC, 20 ºC, 25 ºC, 30 ºC, 35 ºC or 40 ºC or a temperature that is within a range defined by any two of the aforementioned values.

[00146] In some alternatives, the first, second or third media or additional plurality of mediums comprise antibiotics.

[00147] In some alternatives, the exosomes are isolated from said first, second, and/or third fractions or multiple fractions by a method comprising:

(a) passing the first, second and/or third fractions or multiple fractions through a tissue filter;

(b) performing a first centrifugation of the filtrate collected in (a) to generate a cell pellet and a first supernatant;

(c) performing a second centrifugation on the first supernatant to generate a second supernatant; and

(d) performing a third centrifugation on the second supernatant to generate an exosome pellet; and, optionally,

(e) resuspending the exosomes in a solution.

[00148] In some alternatives, the population of isolated exosomes comprise exosomes having CD63, CD63-A, perforin, Fas, TRAIL or granzyme B Bor a combination thereof. In some alternatives, the population of isolated exosomes comprise exosomes that comprise a signaling molecule. In some alternatives, the population of isolated exosomes comprise exosomes that comprise cytokines, mRNA or miRNA.

[00149] In some alternatives, the method further comprises isolating exosomes by affinity chromatography, wherein affinity chromatography is selective for the removal of exosomes comprising viral antigens, viral proteins, bacterial antigens, or bacterial protein fungal antigens or fungal proteins.

[00150] In some alternatives, the method further comprises isolating exosomes by an alternative or additional affinity chromatography step, wherein the alternative or additional affinity chromatography step is selective for the removal of exosomes comprising inflammatory proteins. In some alternatives, the method further comprises enriching a population of exosomes comprising anti-inflammatory biomolecules.

[00151] In some alternatives, exosomes generated by any one of the embodiments herein are provided. In some alternatives, the exosomes are from ascites fluid, blood or plasma. In some alternatives, the exosomes are from cells from an organ. In some alternatives, the exosomes are from immune cells. In some alternatives, the exosomes are from T-cells or B-cells.

[00152] It will be understood by those of skill within the art that, in general, terms used herein, and especially in the appended claims (e.g., bodies of the appended claims) are generally intended as "open" terms (e.g., the term "including" should be interpreted as "including but not limited to," the term "having" should be interpreted as "having at least," the term "includes" should be interpreted as "includes but is not limited to," etc.). It will be further understood by those within the art that if a specific number of an introduced claim recitation is intended, such

an intent will be explicitly recited in the claim, and in the absence of such recitation no such intent is present. For example, as an aid to understanding, the following appended claims may contain usage of the introductory phrases "at least one" and "one or more" to introduce claim recitations. However, the use of such phrases should not be construed to imply that the introduction of a claim recitation by the indefinite articles "a" or "an" limits any particular claim containing such introduced claim recitation to embodiments containing only one such recitation, even when the same claim includes the introductory phrases "one or more" or "at least one" and indefinite articles such as "a" or "an" (e.g., “a” and/or “an” should be interpreted to mean “at least one” or “one or more”); the same holds true for the use of definite articles used to introduce claim recitations. In addition, even if a specific number of an introduced claim recitation is explicitly recited, those skilled in the art will recognize that such recitation should be interpreted to mean at least the recited number (e.g., the bare recitation of "two recitations," without other modifiers, means at least two recitations, or two or more recitations). Furthermore, in those instances where a convention analogous to “at least one of A, B, and C, etc.” is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (e.g., “ a system having at least one of A, B, and C” would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). In those instances where a convention analogous to “at least one of A, B, or C, etc.” is used, in general such a construction is intended in the sense one having skill in the art would understand the convention (e.g., “ a system having at least one of A, B, or C” would include but not be limited to systems that have A alone, B alone, C alone, A and B together, A and C together, B and C together, and/or A, B, and C together, etc.). It will be further understood by those within the art that virtually any disjunctive word and/or phrase presenting two or more alternative terms, whether in the description, claims, or drawings, should be understood to contemplate the possibilities of including one of the terms, either of the terms, or both terms. For example, the phrase “A or B” will be understood to include the possibilities of “A” or “B” or “A and B.”

6. Examples

First Series of Experiments

6.1. Example 1: Cultivation of human placenta

[00153] Human placenta are received and washed with sterile PBS or saline solution to remove blood. The placenta is then cultivated in vessels as a whole organ in a large container with volume of 500 mL or 1000 mL of DMEM culture media supplemented with antibiotics and 2mM EDTA. In a different alternative, the placenta can be cut into different sizes and placed in the culture container. The cultivation is at 37oC in cell culture incubator with 5% CO2. The cultivation time is 4 hour to 8 hours and the supernatant of the culture is used for isolation of exosomes. New media is added at each harvest time point (e.g., every 8 hours or every 12 hours) and the placenta organ and tissue is cultured for up to at least 5 days.

6.2. Example 2: Isolation and purification of placenta exosomes

[00154] The supernatant of the culture is centrifuged at 3,000g for 30minutes to pellet the cell and tissue debris. The supernatant is then centrifuged at 10,000 g for 1 hour and the pellet (small cell debris and organelles) is discarded. The supernatant is then centrifuged at 100,000 g for 2 hours. The resulted pellet is exosomes. The exosomes pellet can be further purified by the following method: resuspended with different volume of sterile PBS and centrifuged again at 100,000 for 2 hours and the final pellet is then resuspended with sterile PBS. The resuspended exosome is filtered through a syringe filter (0.2um), aliquoted at -80oC at different volumes from 300uL to 1 mL.

[00155] Placental exosomes are characterized by size. Size distribution is analyzed by a nanoparticle tracking assay. Three representative samples of pExo were measured with their size using NanoSight. Each isolate has a mean size of 117, 101, and 96 respectively, consistent with the reported size of exosomes. Results are shown in FIG. 2A – FIG. 2C.

6.3. Example 3: Markers of pExos by FACS Analysis

[00156] Protein markers of pExo were analyzed with MACSPlex Exosome Kit (Miltenyi Biotec, Cat#130-108-813) following the protocol provided by the kit. Briefly, the 120uL of pExo isolates were incubated with 15 uL of exosome capture beads overnight at room temperature overnight. After washing once with 1 mL wash solution, the exosome were incubated with exosome detection reagents CD9, CD63 and CD81 cocktail and incubated for additional 1 hrs. After two washes, the samples were analyzed with FACS (BD Canto 10). There are total 37 proteins markers included in this kit (Table 1) excluding mIgG1 and REA control.

Table 1: List of protein markers used to detect pExo in MACSPlex Exosome Kit

No.	Antibody	Isotype	No.	Antibody	Isotype
22	CD3	mIgG2a	65	CD81	REA
23	CD4	mIgG2a	66	MCSP	mIgG1
24	CD19	mIgG1	67	CD146	mIgG1
32	CD8	mIgG2a	68	CD41b	REA
33	HLA-DRDPDQ	REA	74	CD42a	REA
34	CD56	REA	75	CD24	mIgG1
35	CD105	mIgG1	76	CD86	mIgG1
42	CD2	mIgG2b	77	CD44	mIgG1
43	CD1c	mIgG2a	78	CD326	mIgG1
44	CD25	mIgG1	79	CD133/1	mIgG1κ
45	CD49e	mIgG2b	85	CD29	mIgG1κ
46	ROR1	mIgG1κ	86	CD69	mIgG1κ
52	CD209	mIgG1	87	CD142	mIgG1κ
53	CD9	mIgG1	88	CD45	mIgG2a
54	SSEA-4	REA	89	CD31	mIgG1
55	HLA-ABC	REA	96	REA Control	REA
56	CD63	mIgG1κ	97	CD20	mIgG1
57	CD40	mIgG1κ	98	CD14	mIgG2a
63	CD62P	REA	99	mIgG1 control	mIgG1
64	CD11c	mIgG2b			

[00157] pExo samples were identified to be highly positive for the following protein markers including CD1c, CD9, CD20, CD24, CD25, CD29, CD2, CD3, CD8, CD9, CD11c, CD14, CD19, CD31, CD10, CD41b, CD42a, CD44, CD45, CD19c, CD4, CD15, CD19c, CD4, CD56, CD62P, CD83, CD69, CD81, CD86, CD105, CD133-1, CD142, CD148, HLA-ABC, HLA-DRDPDQ, MSCP, ROR1, SSEA-4. pExo has very low level (2.6%) in CD209. Human placenta perfusate, which is obtained by perfuse the vasculature of placenta with saline solution without cultivation with medium and cell culture incubator, was also used to isolate exosomes and analyzed by the same methods for marker protein expression. The perfusate derived exosomes also express high levels of most of the markers found in pExo, but it has significantly lower CD11c (2.0%), MCSP (3.4%) and SSEA-4 (3.5%) comparing with pExos. pExo also has significantly higher levels of CD142 and CD81 comparing with placenta perfusate exosomes. Umbilical cord blood serum was also used to isolate exosomes and analyzed by the same

methods for parker protei expression. Cord blood serum derived exosomes are also positive in most of the protein markers, but in general shows lower levels of each these marker protein expressions. Specifically, comparing with pExo, cord blood serum exosome has lower levels of CD56 (1.4%), CD3 (0.3%) and CD25 (3.9%). SSEA-4 and MSCP protein expression in cord blood serum is significantly lower than pExo but higher than placenta perfusate exosomes. Cord blood serum exosomes also has higher levels of MSCP protein comparing with pExo. These data indicate that cultivated placenta tissues can generate a unique exosome population comparing with non-cultured placenta and cord blood serum. Results for pExo samples, compared to cord blood serum derived exosomes and placenta perfusate exosomes are shown in FIG. 3A – FIG. 3C and Table 2.

Table 2

Protein Markers of Average Expression (%) on Exosomes from Three Different Sources			
Markers	**Cultivated Placenta (N=12)**	**Placenta Perfusate (N=4)**	**Cord Blood Serum (N=4)**
CD1c	9.80%	25.30%	15.60%
CD20	12.80%	10.80%	11.40%
CD24	61.90%	84.20%	12.50%
CD25	29.20%	26.50%	***3.90%***
CD29	69.80%	82.20%	11.20%
CD2	49.80%	67.20%	10.90%
CD3	12.00%	14.60%	0.40%
CD8	64.90%	86.90%	14.40%
CD9	66.20%	80.40%	10.40%
CD11c	37.90%	***2.00%***	11.50%
CD14	67.20%	29.50%	15.60%
CD19	29.30%	80.90%	8.90%
CD31	61.50%	81.50%	13.40%
CD40	67.30%	81.10%	15.60%
CD41b	64.70%	82.40%	12.50%
CD42a	66.10%	84.60%	13.00%
CD44	66.20%	86.30%	15.60%
CD45	24.70%	23.50%	6.20%
CD49e	60.60%	82.00%	15.30%
CD4	58.60%	77.40%	15.10%
CD56	24.20%	14.40%	***1.40%***
CD62P	64.10%	87.20%	15.60%
CD63	64.90%	81.10%	10.20%

CD69	58.20%	65.80%	11.90%
CD81	56.40%	84.40%	15.60%
CD86	39.50%	17.30%	10.90%
CD105	53.60%	30.40%	10.00%
CD133-1	64.60%	44.20%	12.00%
CD142	67.80%	11.60%	13.30%
CD146	70.00%	79.40%	11.50%
CD209	2.60%	0%	**9.70%**
CD326	66.70%	75.50%	6.80%
HLA-ABC	64.60%	82.30%	13.70%
HLA-DRDPDQ	60.80%	83.30%	12.80%
MCSP	44.60%	***3.40%***	***8.10%***
ROR1	64.20%	86.20%	***14.40%***
SSEA-4	58.80%	***3.50%***	***10.80%***

6.4. Example 4: Cytokines and growth factors of pExo samples

[00158] pExo samples were analyzed for their contents of cytokines with MiltiPlex Luminex kit that includes 41 different cytokines. The following tables show the data of cytokines detected on 15 different pExo preparations. The data shows that pExo contains significant level of cytokines (mean >50 pg/mL) including FGF2, G-CSF, Fractalkine, GDGF-AA/BB, GRO, IL-1RA, IL-8, VEGF, and RANTES. pExo also contains detectable levels of cytokines (5 pg/mL to 49 pg/mL) of other cytokines including EGF, Flt-3L, IFNa3, MCP-3, PDGF-AA, IL-15, sCD40L, IL6, IP-10, MCP-1, MIP-alpha, MIP-1beta, and TNF-alpha.

Table 3: Cytokines detected in pExo preparations

Sample ID (Table 1-1)	EGF	FGF-2	Eotaxin	TGF-a	G-CSF	Flt-3L	GM-CSF	Fractalkine	IFNa2	IFNg	GRO	IL-10	MCP-3	IL-12P40
	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml
3074-E1	2.79	17.11	3.77	<0.55↓	249.56	1.57	0.49	40.25	7.1	0.61	40.44	0.59	5.87	<0.74↓
3315-E1	4.41	290.32	8.47	<0.55↓	64.49	6.83	1.12	83.56	11.3	1.2	108.91	<0.57↓	4.32	<0.74↓
941-E1	1.59	17.11	<3.20↓	<0.55↓	96.52	<0.62↓	<0.42↓	17.66	2.22	0.87	6.6	0.7	0.85	<0.74↓
941-E2	1.59	12.33	<3.20↓	<0.55↓	141.85	<0.62↓	0.45	22.66	5.19	1.01	6.6	0.62	1.59	<0.74↓
988-E1	4.83	56.94	3.25	0.68	441.69	3.74	1.9	83.56	7.83	1.54	36.15	1.21	5.57	<0.74↓
595-E2	12.76	120.53	11.42	2.03	267.84	5.42	2.2	227.72	13.81	1.93	102.16	1.63	4.32	2.66
595-E3	5	30.09	7.45	<0.55↓	247.34	8.21	1.81	110.13	28.61	4.22	17.13	1.11	<0.38↓	2.73
366-E2	6.18	359.37	6.56	1.27	343.71	12.73	1.71	197.68	7.35	1.46	103	2.33	9.97	1.96
405-E2	9.78	318.88	8.72	1.64	148.99	13.34	1.74	338.31	9.06	0.61	114.73	1.98	9.46	1.28
405-E3	7.91	226.62	6.29	0.84	179.5	4.95	1.53	225.33	7.47	0.48	96.86	1.21	6.75	1.73
352-E1	6.18	508.7	7.1	0.92	48.57	22.98	1.78	385.31	14.81	1.91	139.65	1.86	11.19	4.13
352-E2	5.16	483.27	6.29	0.78	72.77	15.12	1.36	251.86	10.68	1.31	109.76	1.14	5.57	2.21
789-E1	13.48	20.08	7.45	2.29	118.38	<0.62↓	0.98	123.46	5.19	<0.46↓	38.51	1.35	3	1.5
789-E2	5.72	24.95	5.83	<0.55↓	159.06	1.1	1.56	61.1	4.16	0.94	24.96	0.88	5.87	<0.74↓
313-E3	3.72	27.58	4.97	<0.55↓	57.57	<0.62↓	0.7	77.5	20.54	1.82	7.44	0.85	<0.38↓	<0.74↓
	EGF	FGF-2	Eotaxin	TGF-a	G-CSF	Flt-3L	GM-CSF	Fractalkine	IFNa2	IFNg	GRO	IL-10	MCP-3	IL-12P40
Mean	6.07	167.59	6.74	1.31	175.86	8.73	1.38	149.74	10.35	1.42	63.53	1.25	5.72	2.28
SD	3.6	181.0	2.1	0.6	114.3	6.7	0.5	115.0	6.9	0.9	47.8	0.5	3.1	0.9

Sample ID (Table 1-2)	MDC	IL-12P70	PDGF-AA	IL-13	PDGF-AB/BB	IL-15	sCD40L	IL-17A	IL-1RA	IL-1a	IL-9	IL-1b	IL-2	IL-3
	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml
3074-E1	8.33	<0.71↓	3.95	1.3	203.83	2.19	0.81	0.41	10.87	0.77	0.87	0.48	<0.42↓	<0.31↓
3315-E1	<7.64↓	1.12	14.16	2.02	314.97	8.37	0.74	0.39	124.57	0.53	1.29	0.65	<0.42↓	<0.31↓
941-E1	<7.64↓	<0.71↓	1.41	1.01	35.31	0.95	1.5	<0.36↓	9.47	0.39	0.36	1.23	<0.42↓	<0.31↓
941-E2	<7.64↓	<0.71↓	3.58	0.97	93.37	1.2	0.81	0.46	3.48	0.69	0.62	7	<0.42↓	<0.31↓
988-E1	<7.64↓	0.94	8.48	1.59	127	3.76	3.94	0.64	53.63	2.02	0.86	0.79	<0.42↓	<0.31↓
595-E2	8.57	3.07	21.5	4.25	506.7	4.66	25.66	0.95	92.19	2.7	1.84	8.2	<0.42↓	<0.31↓
595-E3	11.62	1.65	12.6	3.9	317.14	3.72	2.65	0.98	18.86	2.09	2.92	3.37	0.53	0.51
366-E2	19.46	1.65	23.19	1.49	439.81	8.44	22.25	0.9	110.52	2.55	0.99	1.39	<0.42↓	0.37
405-E2	45.61	3.2	26.94	1.49	510.45	12.35	23.9	0.5	116.59	1.6	1.08	1.29	<0.42↓	<0.31↓
405-E3	24.28	1.16	18.87	1.28	335.8	10.21	10.81	<0.36↓	90.59	1.12	0.84	1.68	<0.42↓	<0.31↓
352-E1	27.1	3.2	33.76	2.04	492.97	33.13	18.13	1.01	169.21	2.48	1.49	1.5	0.45	<0.31↓
352-E2	14.83	2.14	28.14	1.21	442.07	23.78	11.72	0.98	107.61	1.87	1.18	1.38	<0.42↓	<0.31↓
789-E1	<7.64↓	1.86	7.02	1.61	192.21	2.19	10.47	0.77	91.16	1.71	0.78	0.39	<0.42↓	<0.31↓
789-E2	9.75	1.97	9.3	1.03	210.26	1.8	0.94	0.64	18.86	1.25	0.91	0.64	<0.42↓	<0.31↓
313-E3	<7.64↓	0.94	5.01	2.89	167.09	0.95	<0.56↓	0.85	<3.20↓	1.05	2.93	0.35	<0.42↓	0.42
	MDC	IL-12P70	PDGF-AA	IL-13	PDGF-AB/BB	IL-15	sCD40L	IL-17A	IL-1RA	IL-1a	IL-9	IL-1b	IL-2	IL-3
Mean	**18.84**	**1.91**	**14.53**	**1.87**	**292.60**	**7.85**	**9.55**	**0.73**	**72.69**	**1.52**	**1.26**	**2.02**	**0.49**	**0.43**
SD	12.2	0.8	10.2	1.0	159.1	9.3	9.5	0.2	52.9	0.8	0.8	2.4	0.1	0.1

Sample ID (Table 1-3)	IL-4	IL-5	IL-6	IL-7	IL-8	IP-10	MCP-1	MIP-1a	MIP-1b	RANTES	TNFa	TNFb	VEGF
	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml
3074-E1	<3.20↓	<0.21↓	2.92	2.9	72.66	7.5	17.08	2.24	1.51	143.77	5.64	0.41	21.6
3315-E1	<3.20↓	<0.21↓	6.3	6.2	215.72	27.63	85.9	11.98	8.27	292.91	2.1	0.44	56.06
941-E1	<3.20↓	0.27	1.15	1.45	6.08	<1.30↓	1.67	<1.31↓	<0.33↓	48.16	2.67	0.49	39.7
941-E2	<3.20↓	<0.21↓	1.46	5.34	6.6	<1.30↓	1.53	1.48	0.89	30.32	16.58	0.38	43.8
988-E1	<3.20↓	0.27	9.07	3.6	58.25	47.16	20.48	2.95	2.99	396.33	25.8	0.59	59.12
595-E2	<3.20↓	0.22	20.55	10.12	192.31	14.05	63.62	13.25	3.74	4462	23.97	0.41	51.98
595-E3	<3.20↓	1.39	10.06	6.49	60.01	6.75	11.42	6.76	1.51	265.85	16.15	0.58	106.17
366-E2	5.54	0.47	15.93	4.55	103.91	101.77	71.51	21.83	11.8	2413	5.41	1.73	64.19
405-E2	4.01	0.38	17.02	5	105.05	92.1	99.23	28.81	16.62	2463	6.35	0.97	54.71
405-E3	<3.20↓	0.32	13.3	3.6	159.18	53.34	59.98	27.54	17.63	1655	5.82	0.73	44.31
352-E1	6.08	0.45	24.21	7.24	167.95	156.45	138.26	9.99	8.19	3000	3.29	1.12	67.45
352-E2	<3.20↓	0.38	18.92	5.4	198.95	89.91	103.45	12.06	6.89	2415	2.96	0.76	62.53
789-E1	<3.20↓	0.36	2.62	3.01	17.58	5.64	8.44	1.82	0.85	659.52	7.28	0.55	24.19
789-E2	<3.20↓	0.27	5.69	2.85	84.19	4.65	8.74	3.7	1.04	417.14	5.5	0.52	22.25
313-E3	<3.20↓	0.61	1.11	10.52	8.32	3.67	4.2	3.33	0.53	189.04	3.83	0.52	60.41
	IL-4	IL-5	IL-6	IL-7	IL-8	IP-10	MCP-1	MIP-1a	MIP-1b	RANTES	TNFa	TNFb	VEGF
Mean	**5.21**	**0.45**	**10.02**	**5.22**	**97.12**	**46.97**	**46.37**	**10.55**	**5.88**	**1256.74**	**8.89**	**0.68**	**51.90**
SD	1.1	0.3	7.8	2.6	74.1	49.1	45.2	9.4	5.9	1380.0	7.8	0.4	21.4

[00159] pExo (11 samples) were also analyzed for the presence of soluble cytokine receptors by Multiplex Luminex analysis. The data are shown in the following table. The data shows that pExo contains high levels (>100 pg/mL) of sEFGR, sgp-130, sIL-1R1, sTNFR1, sTNFRII, sVEGRR1, sVEGFR1, sVEGFR3 and sCD30, sIL-2Ra, sIL-6R, sRAGE are also detected in some samples (>10ng/mL). Data shown as < are not detected and are regarded as negative.

Table 4

Soluble cytokine receptors in placenta exosomes																
Location	pExo Samples	sCD30 pg/ml	sEGFR pg/ml	sgp-130 pg/ml	sIL1-RI pg/ml	sIL-1RII pg/ml	sIL-2Ra pg/ml	sIL-4R pg/ml	sIL-6R pg/ml	sRAGE pg/ml	sTNFRI pg/ml	sTNFRII pg/ml	sVEGFR1 pg/ml	sVEGFR2 pg/ml	sVEGFR3 pg/ml
1E3	988E1	11.76	9626	326.15	<12.67↓	<35.04↓	8.5	<59.19↓	10.54	29.49	90.11	14.82	6556	75.84	462.01
1H3	595E1	<6.77↓	3535	209.91	<12.67↓	<35.04↓	<5.97↓	<59.19↓	9.78	8.23	29.88	<12.55↓	2959	<70.59↓	47.7
1C4	941E2	11.41	57601	132.85	<12.67↓	<35.04↓	6.57	<59.19↓	2.85	11.2	34.07	19.66	4929	<70.59↓	119.96
1F4	941E1	8.03	>1003047↑	83.08	<12.67↓	<35.04↓	<5.97↓	<59.19↓	3.65	7.57	25.57	<12.55↓	803.42	<70.59↓	56.61
1A5	405E1	12.84	5863	2316	<12.67↓	206.01	11.6	<59.19↓	197.58	17.06	253.83	365.51	15179	436.1	64.11
1D5	366E1	9.34	10444	2806	<12.67↓	250.03	21.23	<59.19↓	232.91	26.43	322.4	551.01	13823	419.27	101.75
1G5	354E2	19.12	10627	4461	<12.67↓	327.31	17.59	<59.19↓	172.5	19.44	249.47	308.79	19094	1378	86.58
1B6	352E1	14.68	7824	4108	<12.67↓	474.46	16.59	<59.19↓	183.25	13.31	297.87	473.55	16528	908.93	64.11
1E6	789E1	<6.77↓	25357	174.96	<12.67↓	<35.04↓	<5.97↓	<59.19↓	7.18	11.93	28.6	15.44	3144	<70.59↓	273.09
1H6	789E2	<6.77↓	2499	206.92	<12.67↓	<35.04↓	<5.97↓	<59.19↓	6.55	9.86	19.33	<12.55↓	6056	<70.59↓	56.2
1C7	789E3	<6.77↓	2149	197.21	<12.67↓	<35.04↓	<5.97↓	<59.19↓	4.05	6.12	15.19	<12.55↓	9180	<70.59↓	53.33
	Mean	**12.45**	**13552.50**	**1365.64**	**NA**	**314.45**	**13.68**	**NA**	**75.53**	**14.60**	**124.21**	**249.83**	**8931.95**	**643.63**	**125.95**
	SD	**3.66**	**16858.13**	**1725.87**	**NA**	**117.87**	**5.70**	**NA**	**97.06**	**7.72**	**127.23**	**231.22**	**6238.32**	**506.33**	**128.64**
1A3	QC1	465.86	2089	412.75	408.59	1948	420.27	200.54	138.73	252.82	201.08	210.06	4969	1984	1921
1C3	QC2	4219	18434	4012	4060	17200	4021	2290	1996	2165	2013	2005	18121	15711	18072

6.5. Example 5: Proteomic analysis of placenta exosomes

[00160] Three pExo samples were subjected to proteomic analysis. Submitted samples were lysed using a sonic probe (QSonica) with the following settings: amplitude 40%, pulse10x 1 second on, 1 second off. The protein concentration was determined by Qubit fluorometry. 10ug of each sample was processed by SDS page and purified proteins were subject to trypsin digestion. Table 5 shows the total protein identified from each sample. Among these samples, there are total of 1814 proteins identified. Table 6 shows identification and gene ID of top identified proteins in pExo samples. Additional data is shown in FIG. 4 and FIG. 5.

Table 5

	32112	32113	32114
Total number of proteins identified	1313	1130	1362
Total number of spectra matching	22408	20850	23248
Total number of unique peptides	12014	10761	13380

Table 6

Identified Proteins (1814) by Proteomics in Placental Exosomes	Accession Number	Average Relative Abundance
Cytoplasmic aconitate hydratase OS=Homo sapiens GN=ACO1 PE=1 SV=3	sp|P21399|ACOC_HUMAN	**145**
Cell surface glycoprotein MUC18 OS=Homo sapiens GN=MCAM PE=1 SV=2	sp|P43121|MUC18_HUMAN	**131**
Protein arginine N-methyltransferase 1 OS=Homo sapiens GN=PRMT1 PE=1 SV=2	sp|Q99873|ANM1_HUMAN	**119**
Guanine nucleotide-binding protein G(s) subunit alpha isoforms XLas OS=H sapiens GN=GNAS PE=1 SV=2	sp|Q5JWF2|GNAS1_HUMAN	**99**
Cullin-5 OS=Homo sapiens GN=CUL5 PE=1 SV=4	sp|Q93034|CUL5_HUMAN	**91**
Calcium-binding protein 39 OS=Homo sapiens GN=CAB39 PE=1 SV=1	sp|Q9Y376|CAB39_HUMAN	**83**
Glucosidase 2 subunit beta OS=Homo sapiens GN=PRKCSH PE=1 SV=2	sp|P14314|GLU2B_HUMAN	**72**
Chloride intracellular channel protein 5 OS=Homo sapiens GN=CLIC5 PE=1 SV=3	sp|Q9NZA1|CLIC5_HUMAN	**72**
Semaphorin-3B OS=Homo sapiens GN=SEMA3B PE=2 SV=1	sp|Q13214|SEM3B_HUMAN	**72**
60S ribosomal protein L22 OS=Homo sapiens GN=RPL22 PE=1 SV=2	sp|P35268|RL22_HUMAN	**72**
Spliceosome RNA helicase DDX39B OS=Homo sapiens GN=DDX39B PE=1 SV=1	sp|Q13838|DX39B_HUMAN	**71**
Transcriptional activator protein Pur-alpha OS=Homo sapiens GN=PURA PE=1 SV=2	sp|Q00577|PURA_HUMAN	**68**
Programmed cell death protein 10 OS=Homo sapiens GN=PDCD10 PE=1 SV=1	sp|Q9BUL8|PDC10_HUMAN	**66**
BRO1 domain-containing protein BROX OS=Homo sapiens GN=BROX PE=1 SV=1	sp|Q5VW32|BROX_HUMAN	**66**
Kynurenine--oxoglutarate transaminase 3 OS=Homo sapiens GN=KYAT3 PE=1 SV=1	sp|Q6YP21|KAT3_HUMAN	**65**
Laminin subunit alpha-5 OS=Homo sapiens GN=LAMA5 PE=1 SV=8	sp|O15230|LAMA5_HUMAN	**64**
ATP-binding cassette sub-family E member 1 OS=Homo sapiens GN=ABCE1 PE=1 SV=1	sp|P61221|ABCE1_HUMAN	**61**
Syntaxin-binding protein 3 OS=Homo sapiens GN=STXBP3 PE=1 SV=2	sp|O00186|STXB3_HUMAN	**60**
Proteasome subunit beta type-7 OS=Homo sapiens GN=PSMB7 PE=1 SV=1	sp|Q99436|PSB7_HUMAN	**60**
Glycogen [starch] synthase, muscle OS=Homo sapiens GN=GYS1 PE=1 SV=2	sp|P13807|GYS1_HUMAN	**59**

Identified Proteins (1814) by Proteomics in Placental Exosomes	Accession Number	Average Relative Abundance
NAD(P)H-hydrate epimerase OS=Homo sapiens GN=NAXE PE=1 SV=2	sp|Q8NCW5|NNRE_HUMAN	**59**
Hypoxia up-regulated protein 1 OS=Homo sapiens GN=HYOU1 PE=1 SV=1	sp|Q9Y4L1|HYOU1_HUMAN	**57**
Coagulation factor XI OS=Homo sapiens GN=F11 PE=1 SV=1	sp|P03951|FA11_HUMAN	**57**
Histone H1.0 OS=Homo sapiens GN=H1F0 PE=1 SV=3	sp|P07305|H10_HUMAN	**56**
COP9 signalosome complex subunit 4 OS=Homo sapiens GN=COPS4 PE=1 SV=1	sp|Q9BT78|CSN4_HUMAN	**56**
40S ribosomal protein S15a OS=Homo sapiens GN=RPS15A PE=1 SV=2	sp|P62244|RS15A_HUMAN	**56**
Protein ABHD11 OS=Homo sapiens GN=ABHD11 PE=1 SV=1	sp|Q8NFV4|ABHDB_HUMAN	**54**
Retinal dehydrogenase 1 OS=Homo sapiens GN=ALDH1A1 PE=1 SV=2	sp|P00352|AL1A1_HUMAN	**53**
GDP-mannose 4,6 dehydratase OS=Homo sapiens GN=GMDS PE=1 SV=1	sp|O60547|GMDS_HUMAN	**53**
Ketosamine-3-kinase OS=Homo sapiens GN=FN3KRP PE=1 SV=2	sp|Q9HA64|KT3K_HUMAN	**53**
Protein/nucleic acid deglycase DJ-1 OS=Homo sapiens GN=PARK7 PE=1 SV=2	sp|Q99497|PARK7_HUMAN	**52**
Nectin-4 OS=Homo sapiens GN=NECTIN4 PE=1 SV=1	sp|Q96NY8|NECT4_HUMAN	**51**
Cdc42-interacting protein 4 OS=Homo sapiens GN=TRIP10 PE=1 SV=3	sp|Q15642|CIP4_HUMAN	**50**
WD repeat-containing protein 61 OS=Homo sapiens GN=WDR61 PE=1 SV=1	sp|Q9GZS3|WDR61_HUMAN	**49**
CD59 glycoprotein OS=Homo sapiens GN=CD59 PE=1 SV=1	sp|P13987|CD59_HUMAN	**47**
Glycine dehydrogenase (decarboxylating), mitochondrial OS=Homo sapiens GN=GLDC PE=1 SV=2	sp|P23378|GCSP_HUMAN	**46**
Guanine nucleotide-binding protein subunit alpha-11 OS=Homo sapiens GN=GNA11 PE=1 SV=2	sp|P29992|GNA11_HUMAN	**43**
Serpin H1 OS=Homo sapiens GN=SERPINH1 PE=1 SV=2	sp|P50454|SERPH_HUMAN	**42**
Alpha-2-antiplasmin OS=Homo sapiens GN=SERPINF2 PE=1 SV=3	sp|P08697|A2AP_HUMAN	**42**
Heterogeneous nuclear ribonucleoprotein U OS=Homo sapiens GN=HNRNPU PE=1 SV=6	sp|Q00839|HNRPU_HUMAN	**42**

Identified Proteins (1814) by Proteomics in Placental Exosomes	Accession Number	Average Relative Abundance
40S ribosomal protein S11 OS=Homo sapiens GN=RPS11 PE=1 SV=3	sp|P62280|RS11_HUMAN	**41**
3-hydroxyacyl-CoA dehydrogenase type-2 OS=Homo sapiens GN=HSD17B10 PE=1 SV=3	sp|Q99714|HCD2_HUMAN	**41**
SH3 domain-binding glutamic acid-rich-like protein 3 OS=Homo sapiens GN=SH3BGRL3 PE=1 SV=1	sp|Q9H299|SH3L3_HUMAN	**40**
Heterogeneous nuclear ribonucleoprotein Q OS=Homo sapiens GN=SYNCRIP PE=1 SV=2	sp|O60506|HNRPQ_HUMAN	**40**
Bone marrow proteoglycan OS=Homo sapiens GN=PRG2 PE=1 SV=2	sp|P13727|PRG2_HUMAN	**39**
Lysosomal alpha-glucosidase OS=Homo sapiens GN=GAA PE=1 SV=4	sp|P10253|LYAG_HUMAN	**39**
Mannan-binding lectin serine protease 1 OS=Homo sapiens GN=MASP1 PE=1 SV=3	sp|P48740|MASP1_HUMAN	**38**
Tubulin alpha-1A chain OS=Homo sapiens GN=TUBA1A PE=1 SV=1	sp|Q71U36|TBA1A_HUMAN	**37**
CD97 antigen OS=Homo sapiens GN=CD97 PE=1 SV=4	sp|P48960|CD97_HUMAN	**35**
V-type proton ATPase subunit B, brain isoform OS=Homo sapiens GN=ATP6V1B2 PE=1 SV=3	sp|P21281|VATB2_HUMAN	**35**
von Willebrand factor A domain-containing protein 5A OS=Homo sapiens GN=VWA5A PE=2 SV=2	sp|O00534|VMA5A_HUMAN	**34**
Integrin alpha-3 OS=Homo sapiens GN=ITGA3 PE=1 SV=5	sp|P26006|ITA3_HUMAN	**34**
Leucine--tRNA ligase, cytoplasmic OS=Homo sapiens GN=LARS PE=1 SV=2	sp|Q9P2J5|SYLC_HUMAN	**34**
Peptidyl-prolyl cis-trans isomerase FKBP3 OS=Homo sapiens GN=FKBP3 PE=1 SV=1	sp|Q00688|FKBP3_HUMAN	**33**
GTP-binding protein SAR1a OS=Homo sapiens GN=SAR1A PE=1 SV=1	sp|Q9NR31|SAR1A_HUMAN	**33**
Ras-related protein Rab-10 OS=Homo sapiens GN=RAB10 PE=1 SV=1	sp|P61026|RAB10_HUMAN	**33**
Immunoglobulin heavy variable 3-30 OS=Homo sapiens GN=IGHV3-30 PE=1 SV=2	sp|P01768|HV330_HUMAN (+1)	**32**
Ubiquitin carboxyl-terminal hydrolase 14 OS=Homo sapiens GN=USP14 PE=1 SV=3	sp|P54578|UBP14_HUMAN	**32**
Mitochondrial-processing peptidase subunit beta OS=Homo sapiens GN=PMPCB PE=1 SV=2	sp|O75439|MPPB_HUMAN	**31**

Identified Proteins (1814) by Proteomics in Placental Exosomes	Accession Number	Average Relative Abundance
Leucyl-cystinyl aminopeptidase OS=Homo sapiens GN=LNPEP PE=1 SV=3	sp|Q9UIQ6|LCAP_HUMAN	**31**
Serine/threonine-protein kinase 10 OS=Homo sapiens GN=STK10 PE=1 SV=1	sp|O94804|STK10_HUMAN	**31**
Protein MON2 homolog OS=Homo sapiens GN=MON2 PE=1 SV=3	sp|Q7Z3U7|MON2_HUMAN	**31**
Complement component C9 OS=Homo sapiens GN=C9 PE=1 SV=2	sp|P02748|CO9_HUMAN	**31**
Heat shock protein beta-6 OS=Homo sapiens GN=HSPB6 PE=1 SV=2	sp|O14558|HSPB6_HUMAN	**31**
Complement component C8 alpha chain OS=Homo sapiens GN=C8A PE=1 SV=2	sp|P07357|CO8A_HUMAN	**31**
Tetratricopeptide repeat protein 37 OS=Homo sapiens GN=TTC37 PE=1 SV=1	sp|Q6PGP7|TTC37_HUMAN	**30**
Gasdermin-E OS=Homo sapiens GN=GSDME PE=1 SV=2	sp|O60443|GSDME_HUMAN	**30**
Acyl-protein thioesterase 1 OS=Homo sapiens GN=LYPLA1 PE=1 SV=1	sp|O75608|LYPA1_HUMAN	**30**
Exportin-1 OS=Homo sapiens GN=XPO1 PE=1 SV=1	sp|O14980|XPO1_HUMAN	**29**
Membrane cofactor protein OS=Homo sapiens GN=CD46 PE=1 SV=3	sp|P15529|MCP_HUMAN	**28**
Hydroxysteroid dehydrogenase-like protein 2 OS=Homo sapiens GN=HSDL2 PE=1 SV=1	sp|Q6YN16|HSDL2_HUMAN	**28**
ATPase ASNA1 OS=Homo sapiens GN=ASNA1 PE=1 SV=2	sp|O43681|ASNA_HUMAN	**27**
Apolipoprotein D OS=Homo sapiens GN=APOD PE=1 SV=1	sp|P05090|APOD_HUMAN	**27**
Tyrosine-protein kinase Lyn OS=Homo sapiens GN=LYN PE=1 SV=3	sp|P07948|LYN_HUMAN	**27**
Eukaryotic translation initiation factor 3 subunit A OS=Homo sapiens GN=EIF3A PE=1 SV=1	sp|Q14152|EIF3A_HUMAN	**27**
Hemopexin OS=Homo sapiens GN=HPX PE=1 SV=2	sp|P02790|HEMO_HUMAN	**27**
Target of Myb protein 1 OS=Homo sapiens GN=TOM1 PE=1 SV=2	sp|O60784|TOM1_HUMAN	**27**
EH domain-containing protein 2 OS=Homo sapiens GN=EHD2 PE=1 SV=2	sp|Q9NZN4|EHD2_HUMAN	**26**
Spectrin beta chain, erythrocytic OS=Homo sapiens GN=SPTB PE=1 SV=5	sp|P11277|SPTB1_HUMAN	**26**
L-lactate dehydrogenase B chain OS=Homo sapiens GN=LDHB PE=1 SV=2	sp|P07195|LDHB_HUMAN	**26**

Identified Proteins (1814) by Proteomics in Placental Exosomes	Accession Number	Average Relative Abundance
Prefoldin subunit 2 OS=Homo sapiens GN=PFDN2 PE=1 SV=1	sp|Q9UHV9|PFD2_HUMAN	**26**
[Pyruvate dehydrogenase[acetyl-transferring]]-phosphatase 1, mito. OS=H sapiens GN=PDP1 PE=1 SV=3	sp|Q9P0J1|PDP1_HUMAN	**26**
Lupus La protein OS=Homo sapiens GN=SSB PE=1 SV=2	sp|P05455|LA_HUMAN	**26**
DnaJ homolog subfamily B member 1 OS=Homo sapiens GN=DNAJB1 PE=1 SV=4	sp|P25685|DNJB1_HUMAN	**26**
Receptor expression-enhancing protein 5 OS=Homo sapiens GN=REEP5 PE=1 SV=3	sp|Q00765|REEP5_HUMAN	**25**
Calpain-1 catalytic subunit OS=Homo sapiens GN=CAPN1 PE=1 SV=1	sp|P07384|CAN1_HUMAN	**25**
2',3'-cyclic-nucleotide 3'-phosphodiesterase OS=Homo sapiens GN=CNP PE=1 SV=2	sp|P09543|CN37_HUMAN	**25**
Myoferlin OS=Homo sapiens GN=MYOF PE=1 SV=1	sp|Q9NZM1|MYOF_HUMAN	**25**
Plasma kallikrein OS=Homo sapiens GN=KLKB1 PE=1 SV=1	sp|P03952|KLKB1_HUMAN	**25**
Monocyte differentiation antigen CD14 OS=Homo sapiens GN=CD14 PE=1 SV=2	sp|P08571|CD14_HUMAN	**24**
Golgin subfamily A member 3 OS=Homo sapiens GN=GOLGA3 PE=1 SV=2	sp|Q08378|GOGA3_HUMAN	**24**
Twinfilin-1 OS=Homo sapiens GN=TWF1 PE=1 SV=3	sp|Q12792|TWF1_HUMAN	**24**
Eukaryotic translation initiation factor 3 subunit M OS=Homo sapiens GN=EIF3M PE=1 SV=1	sp|Q7L2H7|EIF3M_HUMAN	**23**
Niban-like protein 1 OS=Homo sapiens GN=FAM129B PE=1 SV=3	sp|Q96TA1|NIBL1_HUMAN	**23**
Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1 OS=Homo sapiens GN=GNB1 PE=1 SV=3	sp|P62873|GBB1_HUMAN	**23**
Galactoside-binding soluble lectin 13 OS=Homo sapiens GN=LGALS13 PE=1 SV=1	sp|Q9UHV8|PP13_HUMAN	**22**
Integrin beta-1 OS=Homo sapiens GN=ITGB1 PE=1 SV=2	sp|P05556|ITB1_HUMAN	**22**
Prostaglandin E synthase 3 OS=Homo sapiens GN=PTGES3 PE=1 SV=1	sp|Q15185|TEBP_HUMAN	**22**
Isoleucine--tRNA ligase, cytoplasmic OS=Homo sapiens GN=IARS PE=1 SV=2	sp|P41252|SYIC_HUMAN	**22**
Pregnancy-specific beta-1-glycoprotein 1 OS=Homo sapiens GN=PSG1 PE=1 SV=1	sp|P11464|PSG1_HUMAN	**22**

Identified Proteins (1814) by Proteomics in Placental Exosomes	Accession Number	Average Relative Abundance
Adipocyte plasma membrane-associated protein OS=Homo sapiens GN=APMAP PE=1 SV=2	sp|Q9HDC9|APMAP_HUMAN	**22**
Coiled-coil domain-containing protein 93 OS=Homo sapiens GN=CCDC93 PE=1 SV=2	sp|Q567U6|CCD93_HUMAN	**22**
Protein transport protein Sec31A OS=Homo sapiens GN=SEC31A PE=1 SV=3	sp|O94979|SC31A_HUMAN	**21**
COP9 signalosome complex subunit 3 OS=Homo sapiens GN=COPS3 PE=1 SV=3	sp|Q9UNS2|CSN3_HUMAN	**21**
Uridine 5'-monophosphate synthase OS=Homo sapiens GN=UMPS PE=1 SV=1	sp|P11172|UMPS_HUMAN	**21**
Cullin-4B OS=Homo sapiens GN=CUL4B PE=1 SV=4	sp|Q13620|CUL4B_HUMAN	**20**
La-related protein 7 OS=Homo sapiens GN=LARP7 PE=1 SV=1	sp|Q4G0J3|LARP7_HUMAN	**20**
Matrix metalloproteinase-9 OS=Homo sapiens GN=MMP9 PE=1 SV=3	sp|P14780|MMP9_HUMAN	**20**
Hepatocyte growth factor activator OS=Homo sapiens GN=HGFAC PE=1 SV=1	sp|Q04756|HGFA_HUMAN	**20**
AP-2 complex subunit alpha-2 OS=Homo sapiens GN=AP2A2 PE=1 SV=2	sp|O94973|AP2A2_HUMAN	**20**
Plasma protease C1 inhibitor OS=Homo sapiens GN=SERPING1 PE=1 SV=2	sp|P05155|IC1_HUMAN	**20**

6.6. Example 6: RNA analysis of placenta exosomes

[00161] Three pExo samples were analyzed for their RNA profile by sequencing. Briefly, RNA from pExo samples are extracted and covered to cDNA and sequenced. The sequencing data is then compared to the database to identify type and identify of each sequencing data. Table 7 shows the overall profile of RNA sequencing results. The RNA in pExo contains tRNA, microRNA and other category of non-coding RNA. microRNA is the second most abundant RNA in the composition of pEXO samples. A total of 1500 different microRNA have been identified in these three pExo samples. Some commonly present in all three samples and some are uniquely present in one or two of the samples. The gene ID and relatively frequency and abundance of most abundant microRNAs are shown. MicroRNA are known to play important roles in the function of cell-cell communication.

Table 7

Gene_id	Chromosome	% of Total miRNA
hsa-mir-26b	chr2	**6.2606%**
hsa-miR-26b-5p	chr2	**6.2598%**
hsa-mir-26a-2	chr12	**4.1329%**
hsa-mir-26a-1	chr3	**4.1306%**
hsa-miR-26a-5p	chr12	**4.1306%**
hsa-mir-30d	chr8	**2.7200%**
hsa-miR-30d-5p	chr8	**2.7155%**
hsa-mir-100	chr11	**2.3286%**
hsa-miR-100-5p	chr11	**2.3186%**
hsa-mir-21	chr17	**1.5647%**
hsa-miR-21-5p	chr17	**1.5635%**
hsa-mir-22	chr17	**1.2528%**
hsa-miR-22-3p	chr17	**1.2507%**
hsa-mir-99b	chr19	**1.2358%**
hsa-miR-99b-5p	chr19	**1.2230%**
hsa-mir-181a-2	chr9	**1.0593%**
hsa-mir-181a-1	chr1	**1.0014%**
hsa-miR-181a-5p	chr1	**1.0004%**
hsa-mir-199a-2	chr1	**0.6194%**
hsa-mir-199a-1	chr19	**0.6193%**
hsa-mir-199b	chr9	**0.6192%**
hsa-miR-199a-3p	chr1	**0.6173%**
hsa-miR-199b-3p	chr9	**0.6173%**
hsa-mir-517a	chr19	**0.8630%**
hsa-mir-517b	chr19	**0.8625%**
hsa-mir-221	chrX	**0.7610%**
hsa-miR-221-3p	chrX	**0.7607%**
hsa-mir-30a	chr6	**0.7300%**
hsa-miR-517b-3p	chr19	**0.6874%**
hsa-miR-517a-3p	chr19	**0.6873%**
hsa-mir-24-2	chr19	**0.7529%**
hsa-mir-24-1	chr9	**0.7334%**
hsa-miR-24-3p	chr19	**0.7329%**
hsa-mir-512-1	chr19	**0.7532%**
hsa-mir-512-2	chr19	**0.7532%**
hsa-miR-512-3p	chr19	**0.7524%**
hsa-mir-519a-1	chr19	**0.7262%**
hsa-mir-141	chr12	**0.7506%**
hsa-mir-103a-2	chr20	**0.6143%**
hsa-miR-103a-3p	chr20	**0.6130%**

Gene_id	Chromosome	% of Total miRNA
hsa-mir-103a-1	chr5	**0.6130%**
hsa-miR-141-3p	chr12	**0.7479%**
hsa-miR-30a-5p	chr6	**0.6009%**
hsa-mir-200c	chr12	**0.6287%**
hsa-miR-200c-3p	chr12	**0.6286%**
hsa-mir-148a	chr7	**0.3417%**
hsa-miR-148a-3p	chr7	**0.3408%**
hsa-mir-519c	chr19	**0.6193%**
hsa-mir-516b-1	chr19	**0.7180%**
hsa-miR-516b-5p	chr19	**0.7178%**
hsa-mir-518e	chr19	**0.5433%**
hsa-miR-320a	chr8	**0.9335%**
hsa-mir-320a	chr8	**0.9335%**
hsa-mir-522	chr19	**0.5108%**
hsa-mir-23a	chr19	**0.3359%**
hsa-miR-23a-3p	chr19	**0.3356%**
hsa-mir-27b	chr9	**0.3544%**
hsa-miR-27b-3p	chr9	**0.3525%**
hsa-mir-519b	chr19	**0.4531%**
hsa-mir-523	chr19	**0.4546%**
hsa-miR-519a-5p	chr19	**0.4557%**
hsa-mir-517c	chr19	**0.3725%**
hsa-mir-486	chr8	**0.4035%**
hsa-miR-486-5p	chr8	**0.4028%**
hsa-miR-519b-5p	chr19	**0.4490%**
hsa-miR-519c-5p	chr19	**0.4490%**
hsa-miR-522-5p	chr19	**0.4490%**
hsa-miR-523-5p	chr19	**0.4490%**
hsa-miR-518e-5p	chr19	**0.4487%**
hsa-mir-143	chr5	**0.2889%**
hsa-miR-143-3p	chr5	**0.2887%**
hsa-mir-516b-2	chr19	**0.5721%**
hsa-mir-519a-2	chr19	**0.2933%**
hsa-mir-10b	chr2	**0.2067%**
hsa-miR-10b-5p	chr2	**0.2065%**
hsa-miR-519a-3p	chr19	**0.2704%**
hsa-mir-30e	chr1	**0.2635%**
hsa-mir-92a-1	chr13	**0.3218%**
hsa-mir-516a-1	chr19	**0.2681%**
hsa-mir-516a-2	chr19	**0.2681%**
hsa-miR-516a-5p	chr19	**0.2676%**
hsa-let-7a-3	chr22	**0.3538%**

Gene_id	Chromosome	% of Total miRNA
hsa-let-7a-1	chr9	**0.3546%**
hsa-let-7a-5p	chr11	**0.3544%**
hsa-let-7a-2	chr11	**0.3529%**
hsa-mir-424	chrX	**0.2370%**
hsa-miR-92a-3p	chr13	**0.2961%**
hsa-mir-92a-2	chrX	**0.2961%**
hsa-mir-93	chr7	**0.2251%**
hsa-miR-93-5p	chr7	**0.2249%**
hsa-mir-526b	chr19	**0.2720%**
hsa-miR-1323	chr19	**0.3653%**
hsa-mir-1323	chr19	**0.3653%**
hsa-miR-526b-5p	chr19	**0.2701%**
hsa-let-7f-2	chrX	**0.2072%**
hsa-let-7f-5p	chr9	**0.2072%**
hsa-let-7f-1	chr9	**0.2055%**
hsa-miR-517c-3p	chr19	**0.1967%**
hsa-let-7b	chr22	**0.2197%**
hsa-let-7b-5p	chr22	**0.2197%**
hsa-mir-151a	chr8	**0.2002%**
hsa-miR-519c-3p	chr19	**0.1702%**
hsa-mir-148b	chr12	**0.1442%**
hsa-miR-107	chr10	**0.1520%**
hsa-mir-107	chr10	**0.1520%**
hsa-miR-148b-3p	chr12	**0.1411%**
hsa-let-7i	chr12	**0.1502%**
hsa-let-7i-5p	chr12	**0.1502%**
hsa-miR-101-3p	chr1	**0.1174%**
hsa-mir-101-2	chr9	**0.1174%**
hsa-mir-101-1	chr1	**0.1162%**
hsa-miR-424-3p	chrX	**0.1552%**
hsa-mir-519d	chr19	**0.1433%**
hsa-mir-27a	chr19	**0.1629%**
hsa-miR-517-5p	chr19	**0.1751%**
hsa-miR-27a-3p	chr19	**0.1583%**
hsa-mir-23b	chr9	**0.1206%**
hsa-miR-23b-3p	chr9	**0.1205%**
hsa-mir-10a	chr17	**0.0945%**
hsa-miR-10a-5p	chr17	**0.0936%**
hsa-miR-30e-3p	chr1	**0.1370%**
hsa-mir-1283-2	chr19	**0.1558%**
hsa-miR-30e-5p	chr1	**0.1264%**
hsa-miR-30a-3p	chr6	**0.1291%**

Gene_id	Chromosome	% of Total miRNA
hsa-mir-191	chr3	**0.1309%**
hsa-miR-191-5p	chr3	**0.1305%**
hsa-miR-1283	chr19	**0.1416%**
hsa-mir-1283-1	chr19	**0.1416%**
hsa-mir-423	chr17	**0.1596%**
hsa-mir-520a	chr19	**0.1325%**
hsa-miR-151a-3p	chr8	**0.1290%**
hsa-mir-520d	chr19	**0.1287%**
hsa-miR-520d-3p	chr19	**0.1263%**
hsa-miR-520a-3p	chr19	**0.1242%**
hsa-mir-518c	chr19	**0.1092%**
hsa-miR-519d	chr19	**0.1026%**
hsa-mir-335	chr7	**0.0681%**
hsa-mir-524	chr19	**0.1320%**
hsa-mir-16-2	chr3	**0.0867%**
hsa-mir-25	chr7	**0.1007%**
hsa-miR-25-3p	chr7	**0.1005%**
hsa-miR-335-5p	chr7	**0.0645%**
hsa-mir-16-1	chr13	**0.0833%**
hsa-miR-16-5p	chr13	**0.0829%**
hsa-miR-192-5p	chr11	**0.0956%**
hsa-mir-192	chr11	**0.0956%**
hsa-miR-518c-3p	chr19	**0.0930%**
hsa-miR-423-3p	chr17	**0.1019%**
hsa-miR-424-5p	chrX	**0.0818%**
hsa-mir-140	chr16	**0.0914%**
hsa-miR-320b	chr1	**0.1382%**
hsa-mir-320b-2	chr1	**0.1382%**
hsa-mir-320b-1	chr1	**0.1374%**
hsa-miR-140-3p	chr16	**0.0873%**
hsa-miR-518e-3p	chr19	**0.0946%**
hsa-mir-518b	chr19	**0.0883%**
hsa-let-7g	chr3	**0.0762%**
hsa-let-7g-5p	chr3	**0.0762%**
hsa-miR-518b	chr19	**0.0823%**
hsa-miR-222-3p	chrX	**0.0874%**
hsa-mir-222	chrX	**0.0875%**
hsa-miR-524-3p	chr19	**0.1032%**
hsa-miR-20a-5p	chr13	**0.0595%**
hsa-mir-20a	chr13	**0.0595%**
hsa-miR-151a-5p	chr8	**0.0712%**
hsa-miR-186-5p	chr1	**0.0752%**

Gene_id	Chromosome	% of Total miRNA
hsa-mir-186	chr1	**0.0752%**
hsa-mir-660	chrX	**0.0606%**
hsa-miR-660-5p	chrX	**0.0604%**
hsa-mir-125a	chr19	**0.0953%**
hsa-miR-203a	chr14	**0.0536%**
hsa-mir-203a	chr14	**0.0536%**
hsa-mir-106b	chr7	**0.0669%**
hsa-mir-520g	chr19	**0.0731%**
hsa-miR-451a	chr17	**0.0587%**
hsa-mir-451a	chr17	**0.0589%**
hsa-miR-522-3p	chr19	**0.0618%**
hsa-mir-378a	chr5	**0.0840%**
hsa-mir-30b	chr8	**0.0724%**
hsa-miR-181a-2-3p	chr9	**0.0589%**
hsa-mir-181b-2	chr9	**0.0656%**
hsa-miR-378a-3p	chr5	**0.0836%**
hsa-miR-181b-5p	chr1	**0.0650%**
hsa-miR-125a-5p	chr19	**0.0842%**
hsa-mir-584	chr5	**0.0728%**
hsa-miR-584-5p	chr5	**0.0728%**
hsa-miR-29a-3p	chr7	**0.0496%**
hsa-mir-29a	chr7	**0.0497%**
hsa-mir-518a-1	chr19	**0.0680%**
hsa-mir-518a-2	chr19	**0.0680%**
hsa-mir-181b-1	chr1	**0.0616%**
hsa-miR-30b-5p	chr8	**0.0685%**
hsa-miR-518a-3p	chr19	**0.0662%**
hsa-mir-28	chr3	**0.0567%**
hsa-mir-146b	chr10	**0.0609%**
hsa-miR-146b-5p	chr10	**0.0607%**
hsa-miR-520g	chr19	**0.0636%**
hsa-mir-515-1	chr19	**0.0543%**
hsa-mir-515-2	chr19	**0.0543%**
hsa-miR-106b-3p	chr7	**0.0554%**
hsa-mir-30c-2	chr6	**0.0559%**
hsa-mir-30c-1	chr1	**0.0555%**
hsa-miR-30c-5p	chr1	**0.0547%**
hsa-mir-518f	chr19	**0.0510%**

6.7. Example 7: Placenta exosome promotes migration of human dermal fibroblast cells (HDF)

[00162] The cytokine profile shows pExo include chemotactic growth factors, suggesting that pExo should have the function to promote cell migration. To examine this, transwell migration assay was set up as the following: 750uL of DMEM basal medium (without serum) was placed on the bottom chamber of a transwell (24-well) plate, pExo was added at 50 uL. PBS was added at the same volume as control. 1x10e5 HDF were seeded on the top chamber of the transwells (8um pore). After 6 to 24 hours, the cells on the top chamber of the transwell were removed by cotton swab. The transwells are then fixed in solution containing 1% ethanol in PBS, followed by stained with 1% crystal violet dissolved in 1% ethanol-PBS. The migrated cells are visualized with microscope. The data shows the example of results of HDF migrated to the bottom side of the transwell while there was significantly less cell migrated through the well in the PBS control transwell. The study demonstrates that pExo can promote the migration of human dermal fibroblast cells. See, FIG. 6.

6.8. Example 8: Placenta exosomes promote migration of human umbilical cord blood endothelial cells (HUVECs)

[00163] Transwell migration assay was also set up as the following: 750uL of DMEM basal medium (without serum) was placed on the bottom chamber of a transwell (24-well) plate, pExo was added at 50 uL. PBS was added at the same volume as control. 2x10e5 HUVEC expressing GFP proteins were seeded on the top chamber of the transwells (8um pore). After 6 to 24 hours, the migrated wells are visualized directly with an inverted fluorescence microscope (AMG). The study demonstrates that all three pExo sample tested can promote the migration of HUVEC in all three duplicated wells. Complete medium for HUVEC is used as a positive control has significant cell migration and PBS is used as an additional control has significantly less cell migrated through comparing with complete media or pExo tested wells. See, FIG. 7.

6.9. Example 9: Placenta exosomes stimulate proliferation of HUVECs

[00164] Cytokine profiles of pExo shows it has several growth factors (PGDF-AA,BB, VEGF) that are known to be involved in the growth of HUVECs. To examine the effect of pExo on the growth and proliferation of HUVEC. HUVEC expressing GFP were seeded at 1x10e4 cells in 96-well plate (transparent bottom and non-transparent walls) in 100 uL of complete HUVEC growth medium. After seeding for 2 hours, cells were attached to the bottom of the wells. The wells are then added with 25uL of different pExo samples (N=6 per sample). The plate is then evaluated with their fluorescence intensity using a plate reader (Synergy H4, excitation

395nm/emission 509 nm) at day-0 and day-2 after seeding. As shown in Figure 13, Complete media demonstrate higher GFP signals (indicator of cell number) from day-0 to day-2. PBS control, in which the complete medium is 50% diluted, showed slight growth comparing with complete media. All eight different pExo samples all showed higher growth of GFP at day 2. See, FIG. 8.

6.10. Example 10: Placenta exosomes stimulate proliferation and colony formatioin of human CD34+ cells

[00165] To test the effects of pExo on the proliferation of hematopoietic stem cells, human umbilical cord blood CD34+ cells (prepared in house) were thawed and cultured in expansion medium containing a cocktail of SCF, Flt-3, KL (medium A) with 10% FCS-IMDM at 1x10e4/cells per ml (N=4). Culture wells were added with either 25 uL of PBS or 25 uL of pExo samples (two pExo samples tested). After one week of culture, the total cell number of each well was counted and the percentage of CD34+ cells in the culture was evaluated by flow cytometry (FACS) using anti-CD34 antibodies. The total CD34+ cell number is calculated as the total cell number in the well to the % of CD34+ cell in the culture. The results showed both pExo treated culture has significantly higher number of CD34+ cells comparing with PBS control culture. pExo was also tested on their effect on CD34+ cells in a colony forming unit culture (CFU). CFU cultures were established with MethoCult H4434 media (Stem Cell Technologies) and pExo or PBS was added at 50uL/mL. After two weeks of culture, the total CFU number in each 35-mm dish is counted (N=3). The data showed that at the presence of pExo, there are significantly higher number of CFU comparing with PBS control cultures. See, FIG. 9 and FIG. 10.

6.11. Example 11: Inhibition of cancer cell proliferation

[00166] MicroRNA data and cytokine data suggest that pExo have the activities to inhibit cancer cell proliferation. pExo samples was used to examine its effect on the growth of SKOV3 (Human ovarian cancer cell line) in 96-well plate. This SKOV3 cells is engineered to express Luciferase, therefore, measuring the luciferase activity is an index of cell growth. A total of 8 different pExo samples were used. 2000 SKOV3 cells were added to 96-well plate in 100 uL of growth medium (DMEM-10% FCS). 2 hrs later, 40 uL of pExo was added to the well (N=6) and supplemented with 60 uL of growth media. 40 uL of PBS was used as control. The complete medium condition is by adding 100 uL of medium to the wells. After culturing for 2 days in

incubator, the activity of the Luciferase are measured with Luciferase Assay Kit (Promega) by lysed the cells and the Luciferase activity was measured with the Luminescence emission with a plate reader (Synergy H4). The data shows that at each cell concentration, pExo treated culture had significantly less Luminex index comparing with PBS control. This data indicates that pExo inhibited the growth of SKOV3 cells. See, FIG. 11.

[00167] A549 cancer cell line (a human lung carcinoma cancer cell line) was seeded at 1500 cells/well in a 96-well plate (Xiceligence). After seeding 24 hrs, pExo are added at three difference dose (5 uL, 25 and 50 uL) in the growth media (100uL). Same amount of PBS was added as control. The growth of the cells can be monitored from day1 to day3 after seeding through the software that reflect the adherence of the cells on wells. The data showed that at the presence of pExo, the growth of the cells, as shown as normalized cell index, was significantly lower at the presence of pExo comparing with PBS controls. Each of the growth curve is the average cell index from three independent wells. See FIG. 12.

[00168] pExo sample was used to examine its effect on the growth of MDA231 (Human breast cancer cell line) in 96-well plate with different cell doses. This MDA231 cells is engineered to express Luciferase, therefore, measuring the luciferase activity is an index of cell growth. Different cell number of MDA231-Luciferase is seeded to 96-well plates (triplicates) and added with 25 uL of pExo#789. After culturing for 2 days in incubator, the activity of Luciferase is measured with Luciferase Assay Kit (Promega) by lysed the cells and the Luciferase activity was measured with the Luminescence emission with a plate reader (Synergy H4). The data shows that at each cell concentration, pExo treated culture had significantly less Luminex index comparing with PBS control. This data indicates that pExo inhibited the growth of MDA231 cells. See, FIG. 13.

6.12. Example 12: Placenta exosomes modulate activation and differentiation of immune cells

[00169] To examine the effect of pExo on immune cells, human umbilical cord blood T cells were labeled with PKH Fluorescence dye and then incubated with pExo or PHA as stimulation. After culturing in RPMI+10% FCS for 5 days, cells are analyzed with FACS with antibodies that can distinguish total T cells as well as subtypes of different type of T cells including CD4, CD8, CD69, CD27. The data shows that at the presence of pExo, the MFI of CD3+ cells are similar to control culture, indicating that pExo alone do not affect the proliferation activity on the T cells.

At PHA stimulation, the MFI significantly reduced, indicating that the cells proliferated, at the presence of both PHA and pExo, MFI is similar to PHA alone, indicating that the cell proliferation is not affected by the presence of pExo. It was found that CD69+ cells are significantly higher in cells treated with pExo, CD69+ cells significantly increased in CD3+ cells (T cells), indicating that T cell activation was increased by pExo. This observation was found in both cord blood T cells and PBMC cells. In addition, pExo was found to increase the percentage of CD56+ cells (NK) cells in PBMC. See, FIG. 14, FIG. 15, FIG. 16, and FIG. 17.

6.13. Example 13: Yield of Exosomes from Cultivated placenta, placenta perfusate and PRP (cord blood serum)

[00170] Placenta perfusate and PRP (cord blood serum) were isolated by the same method of cultivated human placenta tissues. The table below shows the yield of exosome from the placenta perfusate and PRP are significantly less than cultivated placenta.

Table 8

Yield of exosomes (mg) isolated from Placenta perfusate, PRP and Cultivated Placenta			
Samples/Source	Perfusate	PRP	Cultivated Placenta
1	0.30	0.07	114.7
2	0.02	0.39	88.8
3	0.21	0.67	103.4
4	0.25	0.47	70.0
5	0.36		63.1
6	1.35		97.45
7	0.23		70.46
Mean	**0.39**	**0.40**	**86.84**
SD	0.44	0.25	19.50

Discussion:

[00171] The subject methods are capable of producing large amounts of exosomes with unique and advantageous properties. The exosomes are shown to contain many proteins and RNAs which, due to the demonstrated function of the exosomes are believed to be bioactive. The exosomes express many cell surface markers which may act as binding partners, e.g., as a receptor or ligand, and thereby allow targeting of this biological activity to desired cell types.

[00172] The data presented herein show utility for the exosomes of the for a wide variety of indications such as those described in Table 9.

Table 9

Functional Regeneration Indication Targets of pExo	**Rationales**	**References**
Functional regeneration including but not limiting to: stroke, Spinal cord injury, skin lesions, wound healing, acute and chronic myocardial infarction	pExo contains cytokines and growth factors that are involved in chemotaxis. pExo showed activity of enhance cell migration. pExo showed activity in the stimulation of HUVEC cell proliferation.	
Orthopedic, cosmetic and regenerative medicine applications	pExo contains cytokines and growth factors that are involved in chemotaxis. pExo showed activity of enhance cell migration. pExo showed activity in the stimulation of HUVEC cell proliferation.	
Anti-aging applications	pExo contains cytokines and growth factors that are involved in chemotaxis. pExo showed activity of enhance cell migration. pExo showed activity in the stimulation of HUVEC cell proliferation.	
Hair regeneration	pExo contains cytokines and growth factors that are involved in chemotaxis. pExo showed activity of enhance cell migration. pExo showed activity in the stimulation of HUVEC cell proliferation.	
Organ failure	pExo contains cytokines and growth factors that are involved in chemotaxis. pExo showed activity of	

	enhance cell migration. pExo showed activity in the stimulation of HUVEC cell proliferation.	
Vascular disorders	pExo contains cytokines and growth factors that are involved in chemotaxis. pExo showed activity of enhance cell migration. pExo showed activity in the stimulation of HUVEC cell proliferation.	
Erectile dysfunction	pExo contains VEGF, PDGF, FGF2 which are pro-angiogenesis. Degeneration in the vasculature bed can result in erectile dysfuntion. pExo can enhance angiogenesis.	Xie et al. (2008). Growth factors for therapeutic angiogenesis in hypercholesterolemic erectile dysfunction. Asian J Androl. 10:23-7
Protection for radiation induced wound repair	pExo contains FGF2. FGF2 were demonstrated to have protective effect on radiation-induced healing-impaired wound repair in rats.	Kinoda J. et al. (2018). Protective effect of FGF2 and low molecular-weight heparin/protamine nanoparticles on ratiation-induced healing-impaired wound repair in rats. J. Radiat Res. 59:27-34.
Axonal regeneration and locomotor function recovery after Spinal cord injury	pExo contains FGF2. FGF2 were demonstrated to have the activity to stimulate human dental pulp cells to promote axonal regeneration and locomotor fuction recovery after spinal cord injury.	Nagashima et al. (2017). Sci Rep. Priming with FGF2 stimulates human dental pulp cells to promote axonal regeneration and locomotor function recovery after spinal cord injury. 7:13500.
Liver diseases	pExo contains FGF2. FGF2 were demonstrated to have the activity to stimulate cytoglobin expression and activation of human hepatic stellate cells.	Sato-Matsubara et al. (2017) et al. Fibroblast growth factor-2 regulates cytoglobin expression and activation of human hepatic stellate cells via JNK signaling. J. Biol Chem. 292:18961-18972.
Axonal regeneration and locomotor function recovery after Spinal cord injury	pExo contains FGF2. FGF2 were demonstrated to have the activity to promote nerve regeneration and fuctional recovery after mental nerve crush injury.	Lee et al. (2017). Recombinant human fibroblast growth factor-2 promotes nerve regeneration and functional recovery after mental nerve crush injury. Neural Regen Res. 12:629-636.

Polycystic overy syndrome	pExo contains Fractalkine. Fractalkine were demonstrated to have the activity to restore the expression of StAR and progesterone in granulosa cells from patients with polycystic ovary syndrome.	Huang et al. (2016). Fractalkine restore the decreased expression of StAR and progesterone in granulosa cells from patients with polycystic ovary syndrome. Sci. Rep. 6:26205.
Periodontal regeneration	pExo contains FGF2 and PDGF-BB. FGF2 and PDGF-BB can enhance peridontal diseases.	Li et al. (2018). Evaluation of recombinant human FGF-2 and PDGF-BB in periodontal regeneration: A systemic review and meta-analysis. Sci Rep. 7:65..
Hair growth	pExo contains FGF2 and PDGF-BB, VEGF.	Bak et al. (2018) Human umbilical cord blood mesenchymal stem cells engineered to overexpress growth factors accelerate outcomes in hair growth. Korea J. Physiol Pharmcol. 22:555-566.
Axonal regeneration and locomotor function recovery after Spinal cord injury	pExo contains micro-RNA MIR-26a-5p, which have been implicated in the axon regeneration.	Sun et al. (2018). Network analysis of microRNAs, transcription factors, and target genes involved in axon regeneration. J Zhejiang Univer. Sci. 19:293-304.
Anti Cancer Indication Targets of pExo	**pExo contains anti-tumor micro-RNA below**	
Anti-tumor treatments including all different types of cancers eg. Neuroglioma	microRNA-26b: microRNA (miR)-26b inhibits neuroglioma (U87 glioma cells)	Li YP et al. (2017). Effects of microRNA-26b on proliferation and invatioin of glioma cells and related mechanisms. Mol Med Rep 16:4165-4170.
Anti-tumor treatments including all different types of cancers eg. Colan cancer	microRNA-26b: represses colon cancer cell proliferation	Zhang Y et al (2014). MicroRNA-26b represses colon cancer cell proliferation by inhibiting lymphoid enhancer factor 1 expression. Mol Cancer Ther. 13:1942-51.
Anti-tumor treatments including all different types of cancers: eg. Liver cancer	microRNA-26b-5p: inhibiting human intrahepatic cholangiocarcinoma tumor cell lines RBE and HCCC-9810.	Fan et al. (2018). MicroRNA-26-5p regulates cell proliferation, invasion, and metastasis in human intrahepatic cholangiocarcinoma by targeting S100A7. Oncol Lett. 15:386-392.

Anti-tumor treatments including all different types of cancers. Eg. Blader cancer	microRNA-26-a-5p and microRNA-26b-5p inhibits growth of bladder cancer cells.	Niyamoto et al (2016). Tumor-suppressive miRNA-26a-5p and miR-26-5p inhibit cell aggressiveness by regulating PLOD2 in bladder cancer.
Anti-tumor treatments including all different types of cancers	microRAN-26b-5p inhibits hepatocellular carcinoma	Wang Y et al. (2016). Regulation of proliferation, angiogenesis and apoptosis in hepatocellular carcinoma by miR-26b-5p. Tumor Biol. 37:10965-79.
Anti-tumor treatments including all different types of cancers	mir-22 supppress tumorgenesis in breast cancer	Zhang X et al. (2017). miR-22 suppress tumorigenesis and improves radiosensitivity of breast cancer cells by targeting Sirt1. Biol Res. 50:27.
Anti-tumor treatments including all different types of cancers	mic-22 suppress colon cancer cells	Xia SS et al. (2017). MciroRNA-22 suppresses the growth, migration, and invasion of colorectal cancer celsl through a Sp1 negative feedback loop. Oncotarget. 30:36266-36278.
Anti-tumor treatments including all different types of cancers	MiR-99B and Mir-99-B-5P inhibits metastasis of colorectal cancer cells to liver	Li W et al. (2015). miRNA-99-5p suppresses liver metastasis of colorectal cancer by down-regulating mTOR. Oncotarget 6:24448-62.
Anti-tumor treatments including all different types of cancers	mir-181a and mir-181b suppress human glioma cells trigers growth inhibition, induced apoptosis and inhibited invation in glioma cels.	Shi et al. (2008). Has-mir-181a and has-mir-181b functions as tumor suppressors in human glioma cells. Brain Res. 1236:185-93.
Anti-tumor treatments including all different types of cancers	Mir-199a-2, mir-199-a1, mir-199-B, mir-199A-1p, mir-199b-3p micro RNAs are anti-tumor miR199 family that inhibits cancer cell migration and invation in head and neck cancer	Koshizuka et al. (2017). Regulation of ITGA3 by the anti-tumor miR-199 family inhibits cancer migration and invation in head and neck cancer. Cancer Sci. 108:1681-1692.
Anti-tumor treatments including all different types of cancers	Mir-221 and Mir-221-2p inhibits proliferation of pancreatic cancer cells	Xie et al. (2018) MIR-221 inhibits proliferation of pancreatic cell cells via down regualtion of SOCS3. Eur Rev Med Pharmacol Sci. 22:1914-1921.

Anti-tumor treatments including all different types of cancers	MircoRNA-30a inhibits colorectal cancer metastasis through down-regulation of type 1 insulin-like growth factor receptor	Liu YC et al. (2017) MicroRNA-30a inhibits colorectal cancer metastasis through down regulation of type 1 insulin like growth factor receptor.
Anti-tumor treatments including all different types of cancers	miR-130-a-3p inhibits migration and invation in human breast cancer stem cell-like cells	Kong et al. (2018). MiR-130-3p inhibits migration and invation by regulating RAB5B in human breast cancer stem cell-like cells. Biochem Biophys Res Commun. 501:486-493.
Anti-tumor treatments including all different types of cancers: eg. Breast cancer	miR-24-2 inhibits breast cancer cells growth.	Manvati et al. (2105). miR-24-2 regulates genes in survival pathway and demonstrates potentials in reducing cellular viability in combination with docetaxel. Gene. 10:217-24.
Anti-tumor treatments including all different types of cancers: eg. Pancreatic cancer	miR-24-2 inhibits growth of pancreatic cancer cell lines	Pandita et al. (2015). Combined effect of microRNA, nutraceuticals and drug on pancreatic cancer cell lines. Chem Biol Interact. 233:56-64.
Anti-tumor treatments including all different types of cancers: eg. Pancreatic cancer	microRNA-24-1 inhibits hepatomal cell invasion and metastasis	Liu Y et al. (2017). MicroRNA-24-1 suppress mouse hepatoma cell invasion and metastasis via directly targeting O-GlcNAc transferase. Biomed Pharmacother. 91:731-738.
Anti-tumor treatments including all different types of cancers: eg. Bladder cancer	microRNA-24-1 inhibits cancer cell proliferation.	Inoguchi et al. (2014). Tumour suppressive microRNA-24-1 inhibits cancer cell proliferation through targeting FOXM1 in bladder cancer. FEBS Lett. 588:3170-9
Anti-tumor treatments including all different types of cancers: eg. Small lung cancer	miR-512-P contributes to suppression of metastasis in non-small cell lung cancer	Zhu et al. (2015). Inhibition of RAC1-GEF DOCK3 by miR-512-3p contributes to suppression of metastasis in non small cell lung cancer. Int. J. Biochem Cell Biol. 61:103-14.
Anti-tumor treatments including all	miR-141 inhibits heptacocellular carcinoma	Kim et al. (2018). Tumor-suppressing miR-141- complex loaded tissue-adhesive glue

different types of cancers: eg. Hepatocellular carcinoma		for the locoregional treatment for hepatocellular carcinoma
Anti-tumor treatments including all different types of cancers: eg. Papillary thyroid cancer	Mir-141-3p suppress tumor growth and metastasis	Fang et al (2018). MiR-141-3p suppresses tumor growth and metastasis in Papillary thyroid cancer via targeting Yin Yang 1. Anat Rec (Hoboken). Doi. 10.1002/ar. 23940.
Anti-tumor treatments including all different types of cancers: eg. Papillary thyroid cancer	Mir-141-3p suppress the growth and migration of osteosarcoma cells.	Wang et al. (2108). miR-141-3p is a key negative regulator of the EGFR pathway in osteosarcoma. Onco Targets Ther. 11:4461-4478.
Anti-tumor treatments including all different types of cancers: eg. Papillary thyroid cancer	Mir-148a suppress the growth and migration of prostate cancer	Liu et al. (2018). Long non-coading RNA CCAT1/miR-148a/PKCzeta prevents cell migration of prostate cancer by altering macrophage polarization. Prostate. Doi:10.1002/pro.23716.
Other Indication Targets of pExo		
Wound healing	pExo contains high IL-8. IL-8, also known as neutrophil chemotactic factor, has two primary functions. It induces chemotaxis in target cells, primarily neutrophils but also other granulocytes, causing them to migrate toward the site of infection. IL-8 also stimulates phagocytosis once they have arrived. IL-8 is also known to be a potent promoter of angiogenesis. In target cells, IL-8 induces a series of physiological responses required for migration and	

	phagocytosis, such as increases in intracellular Ca2+, exocytosis (e.g. histamine release), and the respiratory burst.	
Wound healing	pExo contains PDGF-AA/BB: Platelet-derived growth factor (PDGF) is one of numerous growth factors that regulate cell growth and division. In particular, PDGF plays a significant role in blood vessel formation, the growth of blood vessels from already-existing blood vessel tissue, mitogenesis, i.e. proliferation, of mesenchymal cells such as fibroblasts, osteoblasts, tenocytes, vascular smooth muscle cells and mesenchymal stem cells as well as chemotaxis, the directed migration, of mesenchymal cells. Platelet-derived growth factor is a dimeric glycoprotein that can be composed of two A subunits (PDGF-AA), two B subunits (PDGF-BB), or one of each (PDGF-AB). PDGF is a potent mitogen for cells of mesenchymal origin, including fibroblasts, smooth muscle cells and glial cells. In both mouse and human, the PDGF	

	signalling network consists of five ligands, PDGF-AA through -DD (including -AB), and two receptors, PDGFRalpha and PDGFRbeta. All PDGFs function as secreted, disulphide-linked homodimers, but only PDGFA and B can form functional heterodimers	
Anti-inflamamation	pExo contains IL-1RA. IL-1RA is a member of the interleukin 1 cytokine family. IL1Ra is secreted by various types of cells including immune cells, epithelial cells, and adipocytes, and is a natural inhibitor of the pro-inflammatory effect of IL1β. This protein inhibits the activities of interleukin 1, alpha (IL1A) and interleukin 1, beta (IL1B), and modulates a variety of interleukin 1 related immune and inflammatory responses.	

Anti infection, anti HIV, anti virus infection, enhance ment of NK cell cytotoxicity	pExo contains high level of RANTES (CCL5). CCL5 is an 8kDa protein classified as a chemotactic cytokine or chemokine. CCL5 is chemotactic for T cells, eosinophils, and basophils, and plays an active role in recruiting leukocytes into inflammatory sites. With the help of particular cytokines (i.e., IL-2 and IFN-γ) that are released by T cells, CCL5 also induces the proliferation and activation of certain natural-killer (NK) cells to form CHAK (CC-Chemokine-activated killer) cells. It is also an HIV-suppressive factor released from CD8+ T cells.	

Second Series of Experiments

6.14 Example 14: Cultivation of human placenta and isolation of exosomes

[00173] Cultivation of human placenta for exosome isolation: Human placenta are received and washed off the blood with sterile PBS or saline solution. The placenta is then processed to tissue blocks (approximately 1x1x1 cm) in 1000 mL of DMEM culture media supplemented with antibiotics. The placenta tissues are then placed in roller bottle bio-bioreactors and placed in cell culture incubator (humidified) with 5% CO2. The cultivation time varies from 4 hours to 16 hours and the supernatant of the culture is used for isolation of exosomes. New media is added at each harvest time point and the cultured for every 8 hours or 12 hours and up to at least 3 days.

[00174] Isolation and purification of placenta exosomes: The supernatant of the culture is centrifuged at 3,000g for 30 minutes to pellet the cell and tissue debris. The 3000g supernatant were frozen at -80oC freezer for further centrifugation. For further centrifugation, frozen -80oC supernatants are thawed at room temperature or at 4oC. For pooled samples, media supernatant from different placenta donors were mixed together. For single donor, supernatants from a single placenta donor is processed. The thawed 3000g supernatant is then centrifuged at 10,000 g for 1 hour and the pellet (small cell debris and organelles) is discarded. The supernatant is then

centrifuged at 100,000 g for 2 hours. The resulted pellet is then resuspended with sterile PBS aliquoted at -80°C.

6.15 Example 15: Characterization of placental exosomes

[00175] The size of pExo isolated is analyzed by Nano particle tracking assay (performed by Zen Bio Inc). A total of 10 different preparation was is shown. The data shows the "mode" size of pExo is 118 +/- 15 nm (nanometer). Both pooled donor (Lot1 to Lot6) and single donor (Lot 7 to Lot 10)

Table 10

Measurement of pExo Size (Zen Bio)			
pExo Prep	Mean size nM	Mode size nM	Donor
Lot#1	181	144	Pooled
Lot#2	185	118	Pooled
Lot#3	190	127	Pooled
Lot#4	186	134	Pooled
Lot#5	169	101	Pooled
Lot#6	170	117	Pooled
Lot#7	175	99	Single
Lot#8	177	116	Single
Lot#9	182	117	Single
Lot#10	180	103	Single
Mean	180	118	
SD	7	15	

[00176] The protein markers of pExo were analyzed with MACSPlex Exosome Kit (Miltenyi Biotec, Cat#130-108-813) following the protocol provided by the kit. Briefly, the 120uL of pExo isolates were incubated with 15 uL of exosome capture beads overnight at room temperature overnight. After washing once with 1 mL wash solution, the exosome was incubated with exosome detection reagents CD9, CD63 and CD81 cocktail and incubated for additional 1 hrs. After two washes, the samples were analyzed with FACS (BD Canto 10). There are total 37 proteins markers included in this kit (FIG. 18) excluding mIgG1 and REA control.

[00177] pExo samples were analyzed for their contents of cytokines with MultiPlex Luminex kit that includes 41 different cytokines. The following tables shows cytokines and growth factors

from different pExo preparations (pooled or single donors). The data showed pExo different levels of cytokines including FGF2, G-CSF, Fractalkine, PDGF-AA/BB, GRO, IL-1RA, IL-8, VEGF, RANTES, IL-15, IL-4, IL-6, IP-10, MCP-1, MIP-1a, MIP-1b, TNFa. These cytokines and growth factor are known to be involved in cell proliferation, tissue and organ regeneration and have immune-modulation activities.

Table 11

Analyte Sample	IL-15	sCD40L	IL-17A	IL-1RA	IL-1a	IL-9	IL-1b	IL-2	IL-3	IL-4
	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL
pExo-Lo#1	9.13	8.87	0.99	75.55	9.56	3.75	42.44	2.02	<0.42↓	121.08
pExo-Lo#4	7.58	7.93	1.35	50.2	6.72	2.8	37.46	1.61	<0.42↓	122.34
pExo-Lo#6	8.61	10.1	1.38	69.13	8.97	3.71	38.55	1.95	<0.42↓	141.09
pExo-Lo#7	8.43	11.12	0.79	37.91	3.75	3.84	12.73	1.11	<0.42↓	71.89
pExo-Lo#10	7.77	16.14	1.12	41.28	3.44	3.12	22.3	1.15	<0.42↓	134.89
pExo-6607	3.72	6.04	<0.67↓	20.02	1.18	2.04	9.13	<0.45↓	<0.42↓	38.24
Mean	7.54	10.03	1.13	49.02	5.60	3.21	27.10	1.57	#DIV/0!	104.92
SD	1.96	3.47	0.25	20.66	3.34	0.70	14.33	0.43	#DIV/0!	40.76

Analyte Sample	IL-5	IL-6	IL-7	IL-8	IP-10	MCP-1	MIP-1a	MIP-1b	RANTES	TNFa	TNFb	VEGF
	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL
pExo-Lo#1	0.45	1319	41.69	6056	258.3	4625	107.83	41.82	1080	49.23	1.16	62.97
pExo-Lo#4	0.41	1121	38.7	7233	216.36	3341	85.69	36.44	799.41	47.26	1.07	55.64
pExo-Lo#6	0.4	1202	35.48	5241	284.84	3981	112.42	47.83	1105	50.36	1.2	62.21
pExo-Lo#7	0.44	825.34	34.37	4450	65.75	1347	108.9	54.75	929.55	49.1	1.07	64.69
pExo-Lo#10	0.37	1744	40.3	7840	131.61	3488	39.66	18.31	1424	9.17	0.92	68.72
pExo-6607	0.3	788.46	27.81	9194	54.89	1650	25.83	12.68	921.81	4.28	0.66	49.33
Mean	0.40	1166.63	36.39	6669.00	168.63	3072.00	80.06	35.31	1043.30	34.90	1.01	60.59
SD	0.05	351.97	5.04	1755.21	98.71	1302.40	38.09	16.61	217.84	21.90	0.20	6.96

Analyte Sample	EGF	FGF-2	Eotaxin	TGF-a	G-CSF	Flt-3L	GM-CSF	Fractalkine	IFNa2	IFNg
	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml
pExo-6548	4.03	209.91	18.1	3.76	898.64	21.61	41.95	154	30.83	2.12
pExo-6562	5.5	181.56	19.77	2.64	684.85	17.85	28.77	58.55	49.01	2.69
pExo-6565	5.18	208.82	18.1	4.91	1104	30.74	47.12	133.57	32.88	2.28
pExo-6570	4.86	302.96	19.41	0.55	412.08	32.85	29.6	157.38	37.91	2.04
pExo-6726	2.22	80.26	9.11	<0.54↓	107.99	<16.17↓	12.71	58.55	63.44	0.67
pExo-6678	2.22	14.31	10.11	0.78	90.68	<16.17↓	10.35	13.78	46.17	<0.63↓
pExo-6607	8.51	500.5	19.95	3.72	489.73	31.8	39.37	160.76	32.37	2.86
pExo-6680	5.96	161.77	12.71	2.84	599.75	22.82	14.84	106.11	32.88	1.3
pExo-6681	1.44	209.68	17.33	<0.54↓	631.44	21.61	24.4	78.7	31.85	1.44
pExo-6950	5.81	297	17.13	1.1	624.17	21.61	26.55	75.31	35.41	2.04
pExo -lot6726	2.79	414	13.16	<0.54↓	245.78	<16.17↓	10.46	68.56	50.41	1.73
pExo- lot9	9.03	689.59	20.31	4.46	599.6	<16.17↓	49.95	136.99	46.85	2.86
One 100,000g Pellet	**EGF**	**FGF-2**	**Eotaxin**	**TGF-a**	**G-CSF**	**Flt-3L**	**GM-CSF**	**Fractalkine**	**IFNa2**	**IFNg**
Mean	**4.80**	**278.11**	**16.27**	**2.75**	**548.96**	**25.11**	**28.01**	**100.19**	**40.82**	**2.00**
SD	**2.41**	**188.97**	**3.96**	**1.62**	**302.38**	**5.75**	**14.19**	**48.91**	**10.22**	**0.69**
pExo-6950p2	3.51	35.92	7.54	0.55	248.15	<16.17↓	9.87	30.13	21.38	0.91
pExo-Lot3 p3	2.41	<8.09↓	10.84	2.06	272.37	<16.17↓	37.77	9.16	22.44	<0.63↓
pExo-Lot1p2	0.6	<8.09↓	<6.77↓	<0.54↓	44.2	<16.17↓	3.68	16.27	7.96	<0.63↓
pExo-Lot2p3	2.97	<8.09↓	<6.77↓	0.86	114.17	17.85	25.2	55.26	4.42	0.79

Analyte Sample	GRO	IL-10	MCP-3	IL-12P40	MDC	IL-12P70	PDGF-AA	IL-13	PDGF-AB/BB	IL-15
	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml
pExo-6548	2016	4	21.28	8.77	<5.22↓	2.4	18.94	8.85	606.84	7.1
pExo-6562	3610	3.5	12.72	13.5	18.38	<0.92↓	17.12	12.58	191.62	10.24
pExo-6565	1182	3.58	10.74	9.31	27.88	<0.92↓	16.85	7.36	462.11	4.36
pExo-6570	3855	3.5	19.71	9.65	22.86	1.11	6.06	9.51	327.18	6.23
pExo-6726	587.58	1	<4.03↓	1.36	17.37	<0.92↓	10.24	3.82	264.89	<1.14↓
pExo-6678	150.81	<0.85↓	<4.03↓	2.55	<5.22↓	<0.92↓	4.07	3.83	291.66	<1.14↓
pExo-6607	1865	3.33	20.34	12.21	19.16	1.11	15.35	9.3	512.8	5.27
pExo-6680	466.65	2.67	10.23	8.55	28.87	1.27	12.25	8.01	228.15	4.12
pExo-6681	8401	2.67	14.59	8.22	15.58	<0.92↓	4.58	8.55	157.15	8.48
pExo-6950	1433	2.66	16.81	8.33	<5.22↓	1.27	15.68	9.73	466.72	8.47
pExo- lot9	8029	2.84	69.15	13.12	19.85	2.8	13.07	8.94	705.45	7.98
pExo -lot6726	864.66	1.16	9.71	4.28	<5.22↓	<0.92↓	10.39	3.82	296.4	2.12
	GRO	IL-10	MCP-3	IL-12P40	MDC	IL-12P70	PDGF-AA	IL-13	PDGF-AB/BB	IL-15
Mean	3,362.46	**2.71**	**21.13**	**7.75**	**20.36**	**1.65**	**12.38**	**7.88**	512.64	**5.94**
SD	2,618.2	**1.0**	**17.7**	**4.0**	**4.7**	**0.7**	**4.9**	**2.8**	186.8	**2.6**
pExo-6950p2	194.03↓	<0.85↓	<4.03↓	2.92	<5.22↓	<0.92↓	8.5	3.93	399.23	3.67
pExo-Lot3 p3	366.51	0.86	8.83	1.8	<5.22↓	<0.92↓	4.74	5.32	468.28	1.36
pExo-Lot1p2	108.48↓	<0.85↓	5.23	2.47	<5.22↓	<0.92↓	1.07	2.52	141.85	3.15
pExo-Lot2p3	285.27	<0.85↓	12.73	<0.43↓	8.48	<0.92↓	3.52	3.93	377.19	<1.14↓
	GRO	IL-10	MCP-3	IL-12P40	MDC	IL-12P70	PDGF-AA	IL-13	PDGF-AB/BB	IL-15
Mean	3,013.08	**0.88**	**7.93**	**1.73**	**8.48**	**NA**	**4.76**	**4.15**	383.888	**2.35**
SD	2,605.86	**#DIV/0!**	**4.16**	**1.23**	**#DIV/0!**	**NA**	**3.47**	**0.79**	105.666	**0.91**

Analyte Sample	IL-6	IL-7	IL-8	IP-10	MCP-1	MIP-1a	MIP-1b	RANTES	TNFa	TNFb	VEGF
	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml
pExo-6548	363.53	25.01	5031	114.16	1287	33.54	17.37	1308	33.35	0.72	23.43
pExo-6562	181.29	29.86	4849	144.09	1575	18.72	1.94	894.28	14.58	0.72	51.59
pExo-6565	412.54	32.12	4515	177.87	997.38	19.04	3.14	541.21	28.11	0.85	<13.43↓
pExo-6570	297.76	28.68	5913	165.28	1124	94.66	38.89	1398	122.98	0.72	53.97
pExo-6726	12.42	21.93	541.89	191.85	65.81	18.13	3.37	965.7	38.18	0.9	<13.43↓
pExo-6678	18.01	16.4	517.72	45.56	121.87	3.42	<1.38↓	571.83	2.82	0.48	<13.43↓
pExo-6607	844.31	29.67	5749	84.1	1711	23.89	5.5	1283	13.83	1.16	27.78
pExo-6680	105.28	15.82	1589	241.84	660.61	9.78	2.91	624.38	6.11	0.99	<13.43↓
pExo-6681	489.07	31.75	6870	245.06	998.88	69.09	13.5	533.19	69.47	1.69	<13.43↓
pExo-6950	248.24	18.4	2870	426.1	618.19	38.9	8.64	1056	70.75	<0.43↓	23.43
pExo- lot9	851.49	30.83	8587	76.15	1552	22.01	7.75	1150	11.73	1.4	24.54
pExo -lot6726	47.14	27.9	1420	234.16	183.09	36.52	6.28	988.9	47.74	<0.43↓	<13.43↓
	IL-6	IL-7	IL-8	IP-10	MCP-1	MIP-1a	**MIP-1b**	RANTES	TNFa	**TNFb**	VEGF
Mean	321.59	35.86	3873.83	179.62	835.74	32.51	**9.96**	891.12	39.38	**0.91**	38.75
SD	250.32	6.69	2161.21	102.87	567.23	25.81	**10.71**	287.32	16.11	**0.27**	19.55
pExo-6950p2	48.27	11.82	269.28	66.55	88.91	14.94	5.5	312.82	63.28	<0.43↓	<13.43↓
pExo-Lot3 p3	157.74	5.46	760.79	17.73	145.25	10.58	<1.38↓	137.93	36.07	<0.43↓	<13.43↓
pExo-Lot1p2	8.97	3.49	82.36	<3.57↓	20.33	3.88	<1.38↓	19.8	9.42	0.52	<13.43↓
pExo-Lot2p3	63.23	9.5	415	5.62	82.7	7.24	1.64	84.55	30.99	<0.43↓	<13.43↓
	IL-6	IL-7	IL-8	IP-10	MCP-1	MIP-1a	MIP-1b	RANTES	TNFa	TNFb	VEGF
Mean	68.55	7.57	863.86	30.07	78.80	9.23	3.72	338.78	84.36	0.52	#DIV/0!
SD	63.09	3.78	286.97	32.14	51.61	4.76	2.52	125.68	22.15	#DIV/0!	#DIV/0!

6.16 Example 16: In vitro functional activities of placenta exosomes (pExo)

[00178] Placenta exosome promotes proliferation of human renal epithelial cells (FIG. 19). Ten different pExo preparations were used test their effect on the proliferation of human renal epithelial cells in a proliferation assay. In the assay, 5x10e4 cells were seeded in 24-well plate per well and each pExo treatment were tested at three concentrations. After 4 days, cells from each well were harvested and counted. The proliferation fold is calculated vs the input cell number. The data showed all 10 pExo preps stimulated the proliferation of pExo comparing with the basal media. All is equivalent to the 20% of complete media (control media) and some were even higher than the complete media. The data indicate that pExo has the activity to promote growth of human kidney epithelial cells.

[00179] Placenta exosome promotes proliferation of human bronchial tracheal epithelial cells (PBTEC) (FIG. 20). in the following example pExo were used at 4 different concentrations from 1ug/mL to 25 ug/mL in 96-well plate to examine their effect on the proliferation of human primary bronchial tracheal lung epithelial cells (3000 cells/well). After 3-days of treatment, cells proliferation was measured with WST-1 proliferation kit (Sigma). The data shows that pExo promote the proliferation of PBTEC in a dose-dependent manner.

[00180] Placenta exosome promotes proliferation of human dermal fibroblast (HDF) (FIG. 21). in the following example pExo were used at 4 different concentrations from 1ug/mL to 25 ug/mL in 96-well plate to examine their effect on the proliferation of human primary bronchial tracheal lung epithelial cells (3000 cells/well). After 3-days of treatment, cells proliferation was measured with WST-1 proliferation kit (Sigma). The data shows that pExo promote the proliferation of human dermal fibroblast in a dose-dependent manner.

6.17 Example 17: In vivo distribution of placenta exosome (pExo)

[00181] To determine the bio-distribution of pExo in vivo, pExo were labeled with a fluorescent dye (Exo-Glow, SBI Inc) and 300ug of labeled pExo were injected into mice via the tail vein. The distribution of the dye was then observed with whole body live imaging system without sacrificing the animals. Free dye was used as a control. The data showed that the signal of pExo persist in mice significantly higher than the free dye up to 6-day in the mice and the pExo are present in both the upper and lower body of the mice.

[00182] To determine distribution of pExo in different organ and tissues, mice were injected with free Exo-Glow dye or labeled pExo (300ug). 48 hrs after injection, mice were sacrificed and organs were analyzed with ex vivo. The data shows that pExo are in lung, liver, spleen, stomach,

GI track, and femur (bone marrow). Ex vivo analysis of the distribution of the dye in different organs were analyzed by ex vivo imaging.

6.18 Example 18: In vivo activity of pExo in tissue and organ repair

[00183] **Stroke model:** To determine if pExo can have in vivo biological activities, two pExo preparation from two single placenta donor were used for MCAO stroke model as the following illustrated study design. Each animal received three 100ug of pExo at day-1, day-6 and day-11 post induction of stroke induction. PBS (vehicle) is used as control. The rats were evaluated with neurological severity score, stepping test, forelimb placement and body score up to day-35 weekly.

[00184] The neurological function of the animals show that rats with stroke treated with pExo showed improved neuroscore significantly from day-7 to day-35. Other functional tests including body swing, fore-limb placement, stepping test all show significant improvement by both pExo treatment.

[00185] **Hind** limb **ischemia model (HLI):** the functions of pExo for tissue and organ repair was tested in a second mice HLI model in which diabetic mouse were induced with surgery to have hindlimb ischemia. The mice were injected (i.v.) with 100ug at days 1, 6 and 11 post surgery and blood flow of the hind limb were measured at week2 and week4 post-surgery. The results show both pExo treatment improved the blood flow of the forelimb of these animals.

[00186] Anti-aging study: Effects of pExo on aging were determined in 52-week-old male C57BL/6J mice. Endpoints were measures of T lymphocytes, plasma insulin and glucose tolerance, accelerating rotarod test, and clinical chemistry and hematology. Results of the study are forthcoming and are expected to continue to demonstrate in vivo, the anti-aging effects of pExo.

[00187] The rotarod assay was carried out using four EzRod test chambers. For the accelerating rotarod paradigm, mice were given 4 trials with the maximum duration of 3 min and a 30-sec ITI. Each mouse was placed on the EZRod machines and the latency to fall was recorded for all trials. If the mouse fell or 3 min elapsed, the mouse was left in the bottom of EzRod test chamber for 30 sec before starting the next trial.

[00188] For glucose tolerance analysis, Mice were fasted for 4 hours. Blood glucose was measured from the tail tip following removal of ~1 mm of tail. The first drop of blood was checked via glucometer (One-Touch Ultra) for time 0. Blood was also collected from the tail

snip at time 0 and processed into plasma for insulin measurements. Immediately following the time 0 procedures, glucose (20% solution in sterile water) was administered via oral gavage (2 g/kg at 10 ml/kg) and subsequent glucose measurements and blood for insulin were collected at 15, 30, 60 and 120 min following the glucose dose.

[00189] GVHD model: Single or multiple doses of pExo were administered IV to mice receiving 30 million human PBMC intravenously. Effects on GVHD were measured by survival and body weight analysis and cell engraftment was analyzed.

[00190] Based on the anti-aging effects and T cell suppression observed above, PD-L1 and Visfatin kits were used to test pExo samples and data normalized to pg or ng/mg. The results show that pExo contains significant levels of PD-L1 and Visfatin (eNAMPT).

Table 12

Visfatin in pExo (ng/mg)	
pExo-6681	11.1
pExo-6607	4.22
pExo-6570	2.64
pExo-6565	3.42
pExo-6562	4.16
pExo-6584	3.82
Mean	4.89
SD	3.10

Samples	PD-L1 (pg/mg of pExo)
pExo#6570	116.7
pExo#6681	50.4
pExo#6680	91.1
pExo#6562	31.4
pExo#6726-a	29.9
pExo#6726-b	27.8
pExo#8161 (F18)	80.1
pExo#8120	113.8
Mean	66.9
SD	36.5

6.19 Example 19: Treatment of Lung Injury with pExo

[00191] To further evaluate the role of pExo in the treatment of lung injury we evaluated the activity of pExo on proliferation of human primary cells (Pulmonary bronchial/tracheal epithelial cells) and compared DMEM cultivated and PBS cultivated pExo in the cell proliferation assays. Cells were seeded in 96-well plate at 3000 cells/well (n=3), washed with PBS after overnight culture, and treated with or without pExo for 2 days followed by WST assay, data normalized to

Basal medium (BM). The results showed that pExo cultivated from DMEM (6 different donors) and PBS (3 different donors) increase the proliferation of Pulmonary bronchial tracheal epithelial cells (PBTEC). These studies demonstrate that pExo could be used for lung injury diseases such as acute respiratory distress syndrome (ARDS) and / or ventilator induced injury of lung infection patients (e.g. COVID-19 patients).

[00192] To evaluate if pExo increases proliferation in dose dependent manner in human primary cells, cells were seeded in 96-well plate at 3000 cells/well (n=3) followed by a wash with PBS after overnight culture. Cells were treated with increasing concentration of pExo (1 to 25 μg/ml) for 2 days followed by WST assay, data normalized to Basal medium. The results demonstrate that pExo increases proliferation in dose dependent manner in PBTEC, further supporting their role in stimulating treatment and their utility as a treatment for lung injury.

[00193] We next sought to evaluate selected cytokine and chemokine composition with MSD assays and to compare pExo from three different cultivation conditions: DMEM, PBS and Saline (0.89% NaCl). Briefly, Isolated pExo through sequential centrifugation as established before being resuspended in PBS or saline and added to MSD assay following manufacturer's instructions. Data were normalized to pg/mg of pExo according to individual pExo concentration and data are average of the samples tested as shown in the table below.

Table 13

pg/mg of pExo	bFGF	Fractalkine	G-CSF	GM-CSF	GROα	HGF	IFN-α2a	IL-6	IL-7	IL-8	IP-10	MCP-1	MIP-1α	RANTES	TNF-α
pExo by DMEM (n=11)	163.84	302.62	902.26	161.73	2048.50	13810.15	3.17	393.76	1.72	5620.93	42.76	513.71	171.02	1369.96	9.17
pExo by PBS (n=6)	141.89	139.96	123.81	3.82	35.14	3436.43	0.43	10.61	0.69	234.09	5.26	18.70	11.49	813.44	2.42
pExo by Saline (n=5)	119.23	329.27	84.85	6.20	96.14	2608.76	2.02	8.02	2.51	123.72	33.26	45.76	28.43	2408.06	1.61

[00194] The results show that pExo contains each of the examined cytokine and chemokine tested. Hepatocyte growth factor (HGF) has the highest level among these tested molecules DMEM cultivated pExo are more enriched with most of these chemokine and cytokines tested compared to the other cultivation methods. This study demonstrates that pExo contains high level of HGF, which as regenerative activities to many cell types and that pExo derived from DMEM cultivation are more enriched with chemokines and cytokines.

6.20 Example 20: Treatment of Covid-19 induced or ventilator induced lung injuries

[00195] Ventilator-associated lung injury (VALI) is an acute lung injury that develops during mechanical ventilation is also termed ventilator-induced lung injury (VILI). During mechanical ventilation, the flow of gas into the lung will take the path of least resistance. Areas of the lung that are collapsed or filled with secretions will be underinflated, while those areas that are relatively normal will be overinflated. These areas will become overdistended and injured. Another possible ventilator-associated lung injury is known as biotrauma. Biotrauma involves the lung suffering injury from any mediators of the inflammatory response or from bacteremia. Finally, oxygen toxicity contributes to ventilator-associated lung injury through several mechanisms including oxidative stress. VALI is most common in people receiving mechanical ventilation for acute lung injury or acute respiratory distress syndrome (ALI/ARDS). 24 percent of people mechanically ventilated will develop VALI for reasons other than ALI or ARDS. (https://en.wikipedia.org/wiki/Ventilator-associated_lung_injury)

[00196] Preclinical data support that mesenchymal stem cells can be used to treat VILI by promoting tissue repair following VILI. MSCs can reduce the injury related pro-inflammatory response to enhance the host response to bacterial infection. It has been shown that MSCs effects through multiple mechanism including direct cell-cell interaction as well as paracrine dependent resulting from both soluble secreted products and microvesicles or exosomes (Horie and Laffrey. (2016) Recent insights: mesenchymal stromal/stem cell therapy for acute respiratory distress syndrome. F1000Research. (doi:10.12688/f1000research.8217.1)).

[00197] We propose to use placenta exosome (pExo) for the treatment of the Covid-19 induced lung injury or VILI based on the following results:

1. pExo promotes the cell proliferation of human lung bronchial epithelial cells in vitro.
2. pExo contains cytokines composition including HGF, PDGF-BB, FGF2. VEGF that are pro-angiogenesis and pro-regeneration.
3. pExo contains chemokines that can attract the migration of HUVEC, epithelial cells for tissue repair.
4. pExo reduces the oxidation toxicity damage to cells.
5. pExo localize to lung in the preclinical animal model.
6. pExo improves mouse angiogenesis in vivo.

Conclusions

[00198] The results present here indicate that human placenta derived exosomes (pExo) contain important biological activities to stimulate the proliferation of cells derived from different human organ and tissues. In vivo data support that the pExo distribute to different organs including lung, liver, kidney, spleen, bone marrow, GI and stomach in rodent models and likely have similar results when administrated in humans. Administration of pExo in human will bring the biological molecules of pExo to these organs and they will persist in these organs as they do in the rodent models. In two animal models of tissue and organ impairment (Stroke and HLI), pExo showed significant benefit to the recovery of the animal comparing with the control group. These evidences support that pExo will be beneficial for the therapeutics in humans including but not limited to the following diseases or indications:

Table 14

Placenta Exosome (pExo) for the Treatment of Human Diseases			
Demonstrated MOA and Functions of pExo in vitro and in vivo	**Including but not limited to the following targeting organs/patient population**	**Including but not limited to the following indications and diseases**	**Including but not limited to the following routes of administration and/or formula**
pExo improved the mobility, improved glucose tolerance and neurogenesis in aged animals; pExo improves astrocyte proliferation; pExo improves survival of neuron cells; pExo improves recovery of animals in Stroke and HLI models, promotes angiogenesis; pExo contains cytokines and chemokines and anti-aging related protein E-NAMPT.	Aging population	Aged related fragility, aged related diabetics, Alzheimer's diseases; aged related macular degeneration, aged related hearing loss, aged related memory loss, aged related cognitive decline, age related dementia, age related nuclear cataract.	I.V. suspension in saline, I.M, inhalation

Promotes proliferation of hepatic stellate cells; In vivo distribution to liver in animal model; Contains hetocyte growth factor and other growth factors; Reduce the chemical cytotoxicity induced by oxidative stress and chemicals; Contains microRNA such as miR-21-2, mir-26-2b which can promotes liver regeneration.	Liver	Chronic and acute liver diseases; liver cirrhosis; cholestatic liver diseases.	I.V. suspension in saline
Promotes proliferation of fibroblasts and dermal fibroblasts. Contains FGF, VEGF. Promotes angiogenesis in vitro and in vivo. Contains microRNA such as miR-21-2, mir-26-2b.	Skin	Wound healing. Skin wound due to chemical burn, fire burn, diabetic foot ulcer;	Surface application; or I.M. or in combination with placenta derived biomaterials
Promotes proliferation of pulmonary bronchial/tracheal epithelial cells. In vivo distribution to lung in animal model.	Lung	Chronic and acute lung disease; acute lung injury, acute respiratory diseases, chronic obstructive pulmonary diseases (COPD), asthma; lung fibrosis; improve recovery of ventilator induced lung injury	I.V. suspension in saline
Promotes proliferation of renal epithelial cells. In vivo distribution to kidney in animal model. Contains microRNA such as miR-21-2, mir-26-2b which can promote liver regeneration.	Kidney	Acute kidney injury (AKI), Chronic kidney diseases (CKD)	I.V. suspension in saline

Promotes proliferation of astrocytes, improve survival of neuronal cells. pExo improves recovery of stroke mouse model and neuron regeneration in animal model.	Brain, Nerve system	Stroke, spinal cord injury,	I.V., local injection, suspension in saline, or in combination with placenta derived biomaterials
Promotes retinal pigment epithelial cells; Demonstrated immune suppression activities in vivo.	Eye	Chronic and acute eye disease. Such as dry-eye syndrome and diabetic retinopathy.	Local application
pExo distributes to spleen in animal model.	Spleen	Diseases that are associated with enlarged or de-regulated spleen functions such as lupus	I.V. suspension in saline
pExo distributes to femur/bone marrow. pExo promotes proliferation of hematopoietic stem cells (CD34+) cells. pExo contains GM-CSF, G-CSF that can promotes the proliferation of hematopoietic stem cells	Bone marrow	Anemia; Leukopenia; Thrombocytopenia	I.V. suspension in saline
pExo distributes to GI in animal model; pExo improves the survival of GVHD animals and suppress T cell proliferation in vivo; pExo contains immune-regulatory protein PD-L1	GI	Crohn's diseases; auto-immune diseases	I.V. suspension in saline
pExo distributes to heart; pExo improves proliferation of HUVEC, promotes angiogenesis, promote migration of	Heart, Cardiovascular system	Hypertension, Atherosclerosis, Myocardial infarction (MI), Chronic heart failure.	I.V. suspension in saline

HUVEC. pExo improve the recovery of stroke and hind-leg ischemia animal models.			

Equivalents:

[00199] The present disclosure is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the subject matter provided herein, in addition to those described, will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

[00200] Various publications, patents and patent applications are cited herein, the disclosures of which are incorporated by reference in their entireties.

What is Claimed:

1. A method of treating a disease, disorder or condition in a subject comprising administering to the subject a population of exosomes or a composition comprising a population of exosomes, wherein said population of exosomes is positive for CD1c, CD20, CD24, CD25, CD29, CD2, CD3, CD8, CD9, CD11c, CD14, CD19, CD31, CD40, CD41b, CD42a, CD44, CD45, CD49e, CD4, CD56, CD62P, CD63, CD69, CD81, CD86, CD105, CD133-1, CD142, CD146, CD209, CD326, HLA-ABC, HLA-DRDPDQ, MCSP, ROR1, SSEA-4, or combinations thereof.

2. The method of claim 1, wherein said population of exosomes is positive for CD1c, CD20, CD24, CD25, CD29, CD2, CD3, CD8, CD9, CD11c, CD14, CD19, CD31, CD40, CD41b, CD42a, CD44, CD45, CD49e, CD4, CD56, CD62P, CD63, CD69, CD81, CD86, CD105, CD133-1, CD142, CD146, CD209, CD326, HLA-ABC, HLA-DRDPDQ, MCSP, ROR1, and SSEA-4.

3. The method of claim 1, wherein said population of exosomes is positive for CD9, CD29, CD42a, CD62P, CD63, CD81, CD133-1, CD146, HLA-DRP, or combinations thereof.

4. The method of claim 3, wherein said population of exosomes is positive for CD9, CD29, CD42a, CD62P, CD63, CD81, CD133-1, CD146, and HLA-DRP.

5. The method of claim 1, wherein said population of exosomes is positive for 2, 3, 4, 5, 6, 7, 8, 9, 10, or more markers selected from the group consisting of CD1c, CD20, CD24, CD25, CD29, CD2, CD3, CD8, CD9, CD11c, CD14, CD19, CD31, CD40, CD41b, CD42a, CD44, CD45, CD49e, CD4, CD56, CD62P, CD63, CD69, CD81, CD86, CD105, CD133-1, CD142, CD146, CD209, CD326, HLA-ABC, HLA-DRDPDQ, MCSP, ROR1, and SSEA-4.
6. The method of claim 3, wherein said population of exosomes is positive for 2, 3, 4, 5, 6, 7, 8, 9, 10, or more markers selected from the group consisting of CD9, CD29, CD42a, CD62P, CD63, CD81, CD133-1, CD146, and HLA-DRP.

7. The method of any one of claims 1-6, wherein said population of exosomes is CD3-, CD11b-, CD14-, CD19-, CD33-, CD192-, HLA-A-, HLA-B-, HLA-C-, HLA-DR-, CD11c- or CD34-.

8. The method of any one of claims 1-6, wherein said population of exosomes is CD3-, CD11b-, CD14-, CD19-, CD33-, CD192-, HLA-A-, HLA-B-, HLA-C-, HLA-DR-, CD11c- and CD34-.

9. The method of any one of claims 1-8, wherein said population of exosomes comprise non-coding RNA molecules.

10. The method of claim 9, wherein said non-coding RNA molecules are microRNAs.

11. The method of claim 10, wherein said microRNAs are selected from the group consisting of the microRNAs in Table 7, and combinations thereof.

12. The method of claim 10, wherein said microRNAs are selected from the group consisting of hsa-mir-26b, hsa-miR-26b-5p, hsa-mir-26a-2, hsa-mir-26a-1, hsa-miR-26a-5p, hsa-mir-30d, hsa-miR-30d-5p, hsa-mir-100, hsa-miR-100-5p, hsa-mir-21, hsa-miR-21-5p, hsa-mir-22, hsa-miR-22-3p, hsa-mir-99b, hsa-miR-99b-5p, hsa-mir-181a-2, hsa-mir-181a-1, hsa-miR-181a-5p, and combinations thereof.

13. The method of any one of claims 1-12, wherein said population of exosomes comprise a cytokine selected from the group consisting of the cytokines in Table 3 or Table 11, and combinations thereof.

14. The method of any one of claims 1-13, wherein said population of exosomes comprise a cytokine receptor in Table 4, and combinations thereof.

15. The method of any one of claims 1-14, wherein said population of exosomes comprise a protein selected from the group consisting of the proteins in Table 6, and combinations thereof.

16. The method of any one of claims 1-14, wherein said population of exosomes comprise a protein selected from the group consisting of Cytoplasmic aconitate hydratase, Cell surface glycoprotein MUC18, Protein arginine N-methyltransferase 1, Guanine nucleotide-binding protein G(s) subunit alpha, Cullin-5, Calcium-binding protein 39, Glucosidase 2 subunit beta, Chloride intracellular channel protein 5, Semaphorin-3B, 60S ribosomal protein L22, Spliceosome RNA helicase DDX39B, Transcriptional activator protein Pur-alpha, Programmed cell death protein 10, BRO1 domain-containing protein BROX, Kynurenine--oxoglutarate transaminase 3, Laminin subunit alpha-5, ATP-binding cassette sub-family E member 1, Syntaxin-binding protein 3, Proteasome subunit beta type-7, and combinations thereof.

17. The method of any one of claims 1-16, wherein said population of exosomes is a placental-derived population of exosomes.

18. The method of claim 17, wherein said placental-derived population of exosomes is derived from a media of a whole placenta culture.

19. The method of claim 17, wherein said placental-derived population of exosomes is derived from a media of a culture comprising placental lobes or portions of a placenta.

20. The method of claim 17, wherein said placental-derived population of exosomes is derived from a media of a culture comprising placental stem cells, preferably placental-derived adherent cells (PDAC).

21. The method of any one of claims 18-20, wherein the media is selected from the group consisting of a tissue culture media, a saline solution, and a buffered saline solution.

22. The method of any one of claims 1-21, wherein said population of exosomes comprise at least one marker molecule at a level at least two-fold higher than a population of exosomes derived from mesenchymal stem cells, cord blood, or placental perfusate.

23. The method of any one of claims 1-22, wherein said population of exosomes comprise at least one marker molecule at a level at least two-fold lower than a population of exosomes derived from mesenchymal stem cells, cord blood, or placental perfusate.

24. The method of any one of claims 1-23, wherein the disease, disorder or condition is a lung disease disorder or condition.

25. The method of claim 24, wherein the lung disease disorder or condition is selected from the group consisting of acute lung injury, acute and chronic diseases, asthma, chronic obstructive pulmonary disease (COPD), lung fibrosis, idiopathic pulmonary fibrosis, recovery of lung surgery after lung cancer, pulmonary embolism, acute respiratory distress syndrome, pneumonia, viral infection, coronavirus infection, Covid-19, and ventilator induced lung injury.

26. The method of any one of claims 1-23, wherein the disease, disorder or condition is a liver disease disorder or condition.

27. The method of claim 26, wherein the liver disease disorder or condition is selected from the group consisting of acute liver injury, acute and chronic diseases, liver cirrhosis, liver fibrosis, liver inflammation, metabolic disorders, liver damages caused by drugs, poisons, alcohol, virus (e.g., hepatitis) or other infectious disease, and cholestatic liver diseases.

28. The method of any one of claims 1-23, wherein the disease, disorder or condition is a brain / spinal cord disease disorder or condition.

29. The method of claim 28, wherein the brain / spinal cord disease disorder or condition is selected from the group consisting of acute brain / spinal cord injury, acute and chronic diseases, stroke, transient ischemic attach, Parkinson's and other movement disorders, dementias, Alzheimer's diseases epilepsy / seizures, myelopathy, multiple sclerosis, infections of the central nervous system, spinal cord trauma, spinal cord inflammation, amyotrophic lateral sclerosis, spinal muscular atrophy.

30. The method of any one of claims 1-23, wherein the disease, disorder or condition is a kidney disease disorder or condition.

31. The method of claim 30, wherein the kidney disease disorder or condition is selected from the group consisting of acute kidney injury, acute and chronic diseases, kidney injury or damage induced by trauma, drugs (e.g., chemotherapeutic agents), kidney cysts, kidney stones, and kidney infections, recovery of kidney function after kidney transplant, diabetic nephropathy, and polycystic kidney disease.

32. The method of any one of claims 1-23, wherein the disease, disorder or condition is a gastrointestinal disease disorder or condition.

33. The method of claim 32, wherein the gastrointestinal disease disorder or condition is selected from the group consisting of acute gastrointestinal injury, autoimmune disease, acute and chronic diseases, Crohn's disease, irritable bowel syndrome, perianal abscesses, colitis, colon polyps and cancer.

34. The method of any one of claims 1-23, wherein the disease, disorder or condition is a bone marrow disease disorder or condition.

35. The method of claim 32, wherein the bone marrow disease disorder or condition is selected from the group consisting of acute and chronic diseases, anemia, leukopenia, thrombocytopenia aplastic anemia, myeloproliferative disorders, and stem cell transplantation.

36. The method of any one of claims 1-23, wherein the disease, disorder or condition is an eye disease disorder or condition.

37. The method of claim 36, wherein the eye disease disorder or condition is selected from the group consisting of acute eye injury, chronic and acute eye diseases, dry-eye syndrome and diabetic retinopathy, and macular degeneration.

38. The method of any one of claims 1-23, wherein the disease, disorder or condition is a spleen disease disorder or condition.

39. The method of claim 38, wherein the spleen disease disorder or condition is selected from the group consisting of acute spleen injury, chronic and acute spleen diseases, diseases associated with enlarged or de-regulated spleen functions, and lupus.

40. The method of any one of claims 1-23, wherein the disease, disorder or condition is a skin disease disorder or condition.

41. The method of claim 40, wherein the skin disease disorder or condition is selected from the group consisting of acute skin injury, chronic and acute skin diseases, diabetic foot ulcer, wound due to chemical burn, fire burn, skin or tissue damage caused, e.g., by injury, disease or surgical procedures, hair loss, a hair follicle disease, disorder or condition, wrinkles, and reduced firmness.

42. The method of any one of claims 1-23, wherein the disease, disorder or condition is an ischemic disease disorder or condition.

43. The method of claim 42, wherein the ischemic disease disorder or condition is selected from the group consisting of acute ischemic injury, chronic and acute ischemic diseases, ischemic heart disease, ischemic vascular disease, ischemic colitis, mesenteric ischemia, Brain ischemia (e.g., stroke), acute or chronic limb ischemia, cutaneous ischemia, ischemic kidney, and the promotion of angiogenesis in tissues or organs in need thereof.

44. The method of any one of claims 1-23, wherein the disease, disorder or condition is a heart / cardiovascular disease disorder or condition.

45. The method of claim 44, wherein the heart / cardiovascular disease disorder or condition is selected from the group consisting of acute heart / cardiovascular injury, hypertension, atherosclerosis, myocardial infarction (MI), and chronic heart failure.

46. The method of any one of claims 1-23, wherein the disease, disorder or condition is an aging associated disease disorder or condition.

47. The method of claim 42, wherein the ageing associated disease disorder or condition is selected from the group consisting of age related fragility, age related diabetics, Alzheimer's diseases; age related macular degeneration, age related hearing loss, age related memory loss, age related cognitive decline, age related dementia, age related nuclear cataract, age associated loss of function and other effects of ageing.

48. The method of any one of claims 1-23, wherein the disease, disorder or condition is a systemic disease disorder or condition.

49. The method of claim 48, wherein the systemic disease disorder or condition is selected from the group consisting of acute and chronic diseases, graft versus host disease, and infections (e.g., ear infection).

50. The method of any one of claims 1-49, wherein the composition is formulated for intravenous administration.

51. The method of any one of claims 1-49, wherein the composition is formulated for local injection.

52. The method of any one of claims 1-49, wherein the composition is formulated for topical administration.

53. The method of any one of claims 1-49, wherein the composition is formulated for inhalation.

54. The method of any one of claims 1-49, wherein the composition is formulated for oral administration.

55. The method of any one of claims 1-49, wherein the composition is formulated for subcutaneous administration.

56. The method of any one of claims 1-49, wherein the composition is formulated for buccal or sublingual administration.

57. The method of any one of claims 1-49, wherein the composition is formulated for administration to the ear.

58. The method of any one of claims 1-49, wherein the composition is formulated for nasal administration.

59. The method of any one of claims 1-49, wherein the composition is formulated for ocular administration.

60. The method of any one of claims 1-59, wherein the subject is a human.

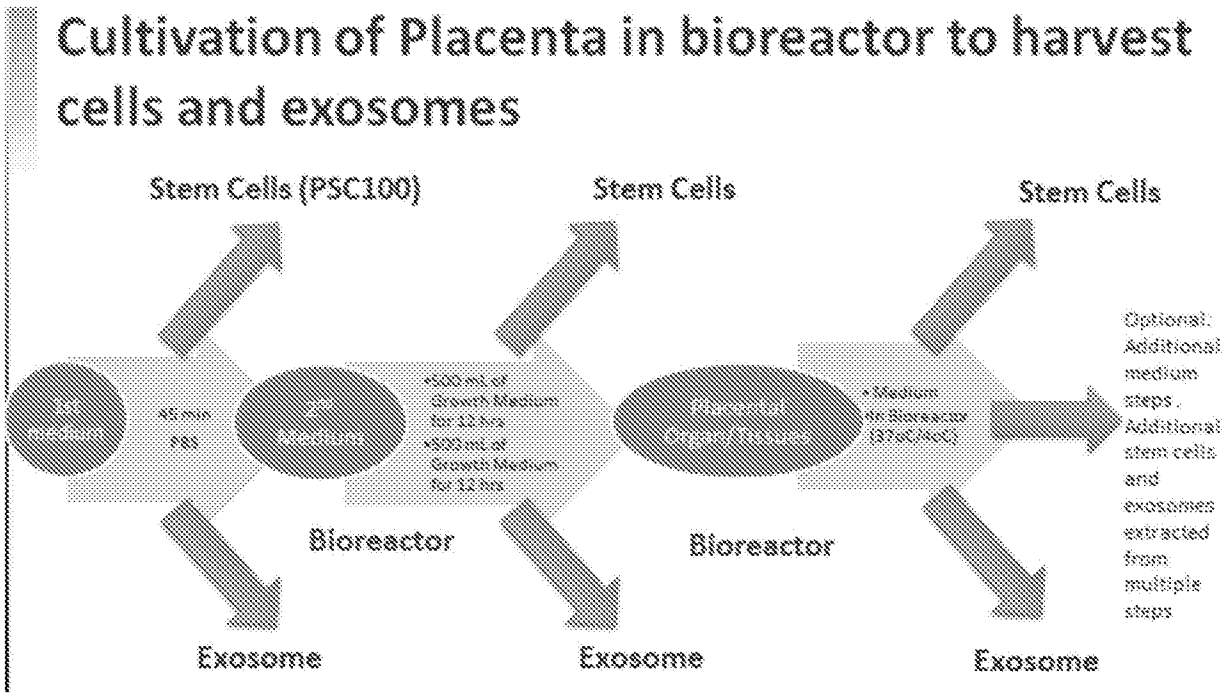

Cultivation of Placenta in bioreactor to harvest cells and exosomes
Stem Cells (PSC100)
Stem Cells
Stem Cells
Bioreactor
Bioreactor
Exosome
Exosome
Exosome
Optional: Additional medium steps . Additional stem cells and exosomes extracted from multiple steps

FIG. 1

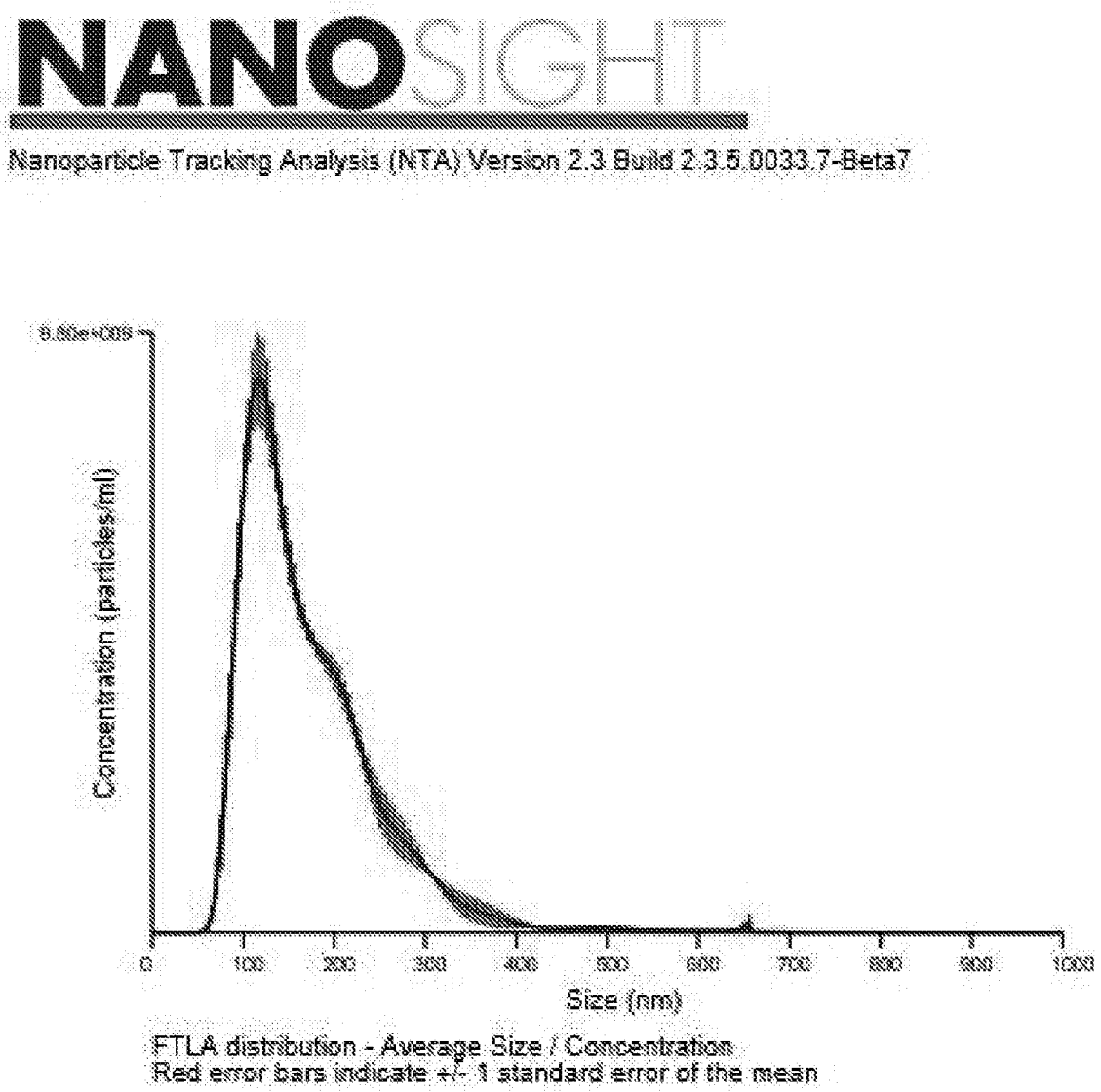
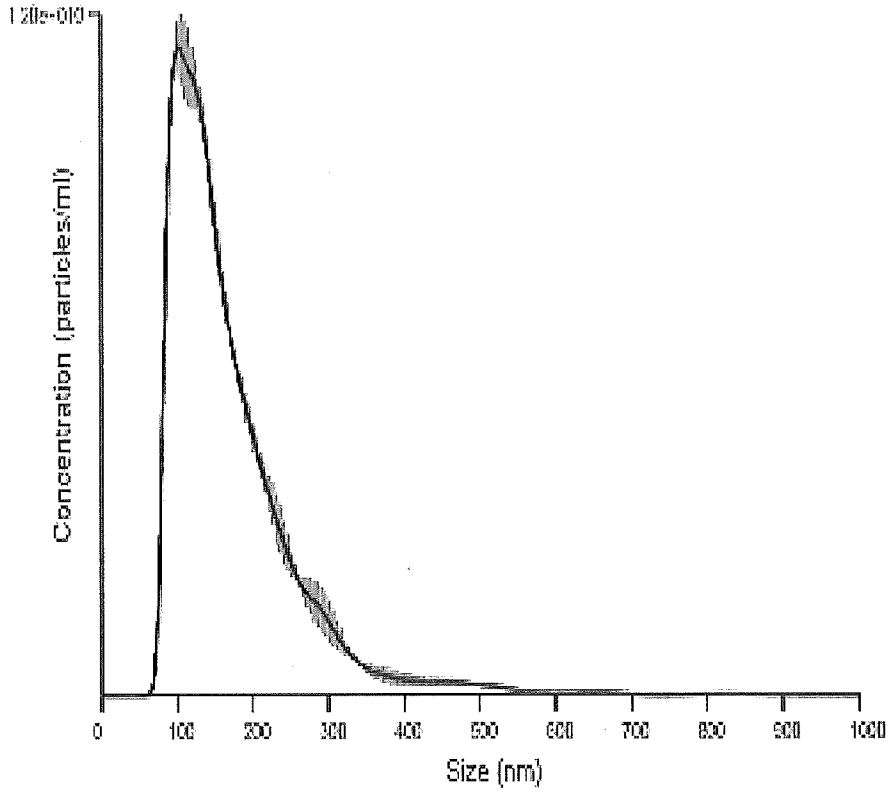
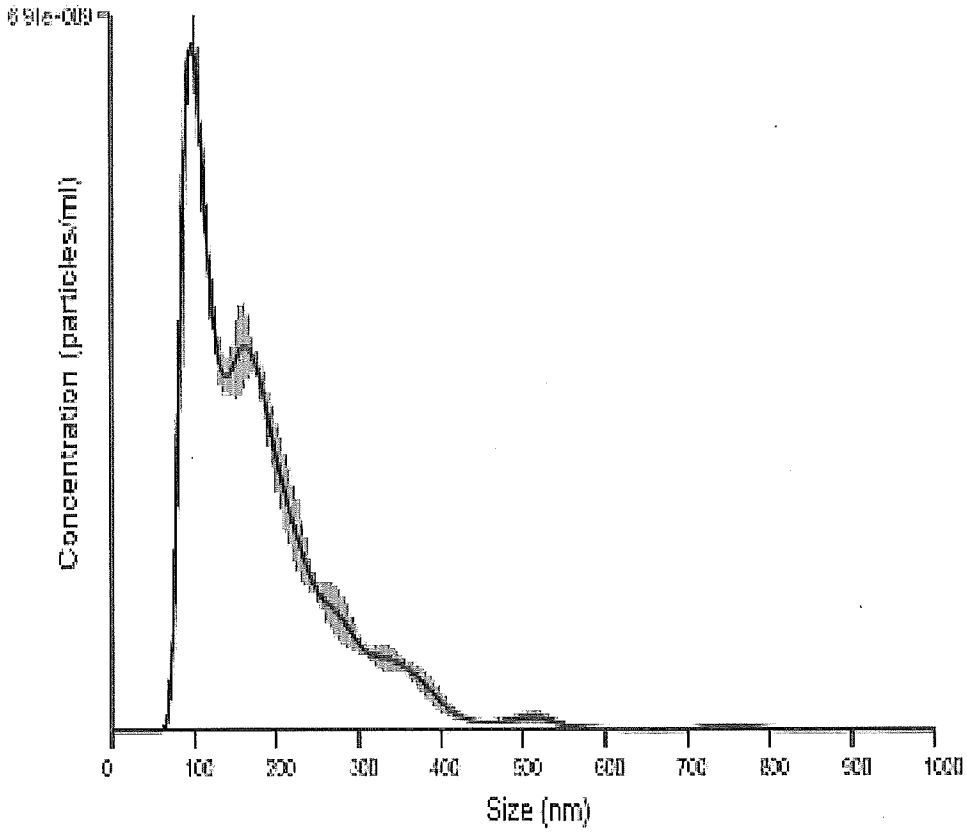

NANOSIGHT
Nanoparticle Tracking Analysis (NTA) Version 2.3 Build 2.3.5.0033.7-Beta7
Concentration (particles/ml)
0
100
200
300
400
500
600
700
800
900
1000
Size (nm)
FTLA distribution - Average Size / Concentration
Red error bars indicate +/- 1 standard error of the mean

FIG. 2A



FTLA distribution - Average Size / Concentration
Red error bars indicate +/- 1 standard error of the mean

FIG. 2B



FTLA distribution - Average Size / Concentration
Red error bars indicate +/- 1 standard error of the mean

FIG. 2C

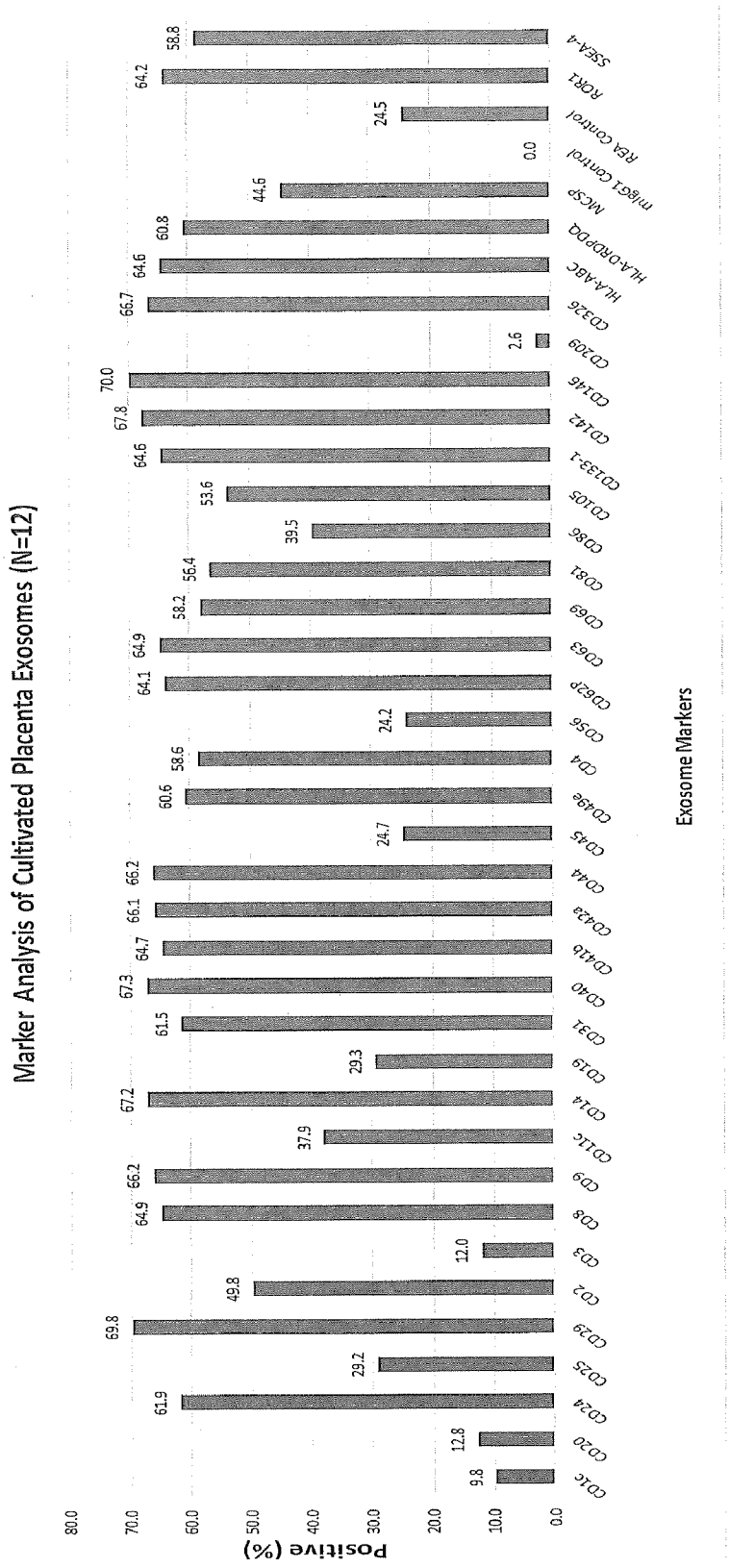

Marker Analysis of Cultivated Placenta Exosomes (N=12)
Positive (%)
Exosome Markers
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
CD1c 9.8
CD20 12.8
CD24 61.9
CD25 29.2
CD29 69.8
CD2 49.8
CD3 12.0
CD8 64.9
CD9 66.2
CD11c 37.9
CD14 67.2
CD19 29.3
CD31 61.5
CD40 67.3
CD41b 64.7
CD42a 66.1
CD44 66.2
CD45 24.7
CD49e 60.6
CD4 58.6
CD56 24.2
CD62P 64.1
CD63 64.9
CD69 58.2
CD81 56.4
CD86 39.5
CD105 53.6
CD133-1 64.6
CD142 67.8
CD146 70.0
CD209 2.6
CD326 66.7
HLA-ABC 64.6
HLA-DRDPDQ 60.8
MCSP 44.6
mIgG1 Control 0.0
REA Control 24.5
RCAS1 64.2
SSEA-4 58.8

FIG. 3A

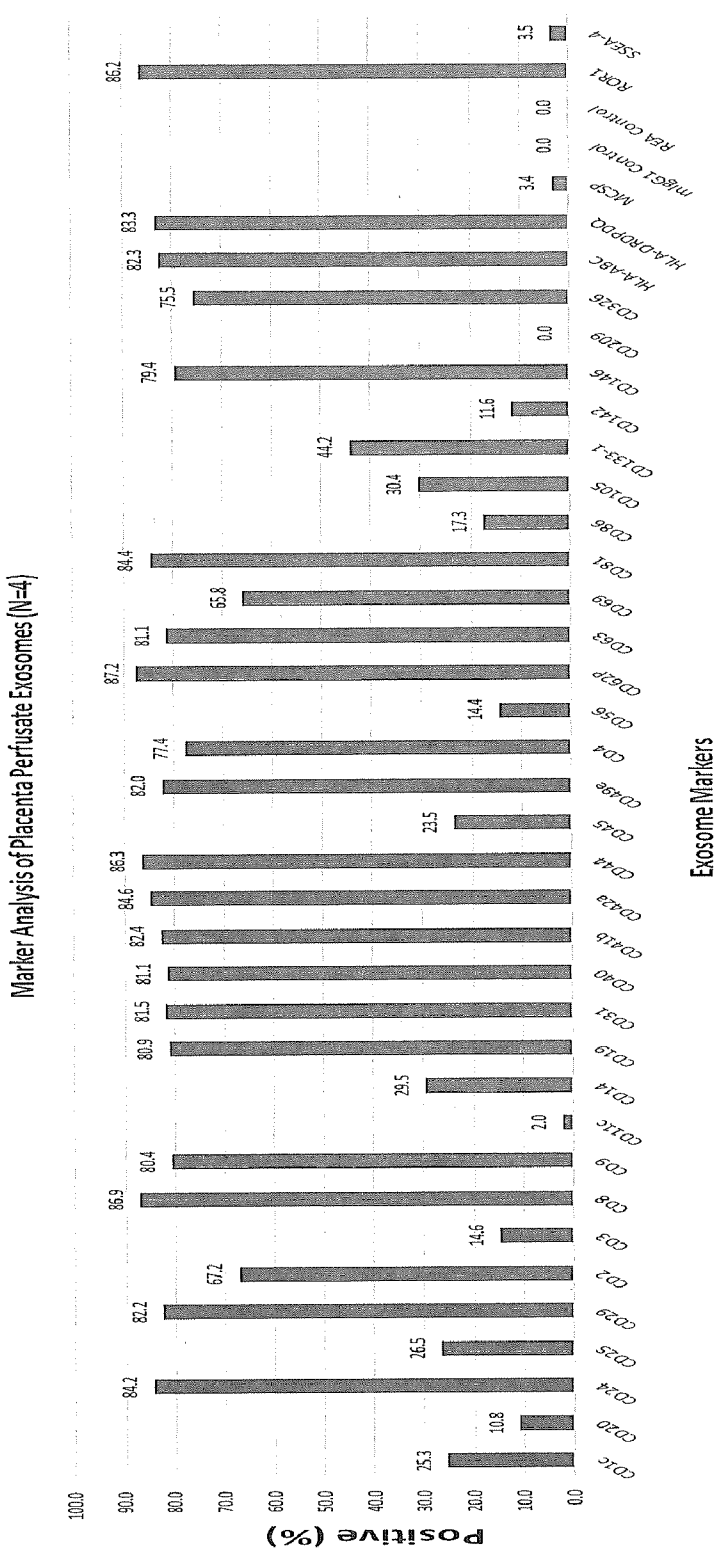

Marker Analysis of Placenta Perfusate Exosomes (N=4)
Positive (%)
Exosome Markers
SSEA-4
RCR1
Rea control
mIgG1 control
MICSP
HLA-DRDPDQ
HLA-ABC
CD326
CD209
CD146
CD142
CD133-1
CD105
CD86
CD81
CD69
CD63
CD62P
CD56
CD4
CD49e
CD45
CD44
CD42b
CD41b
CD40
CD31
CD19
CD14
CD11c
CD9
CD8
CD3
CD2
CD29
CD25
CD24
CD20
CD1c

FIG. 3B

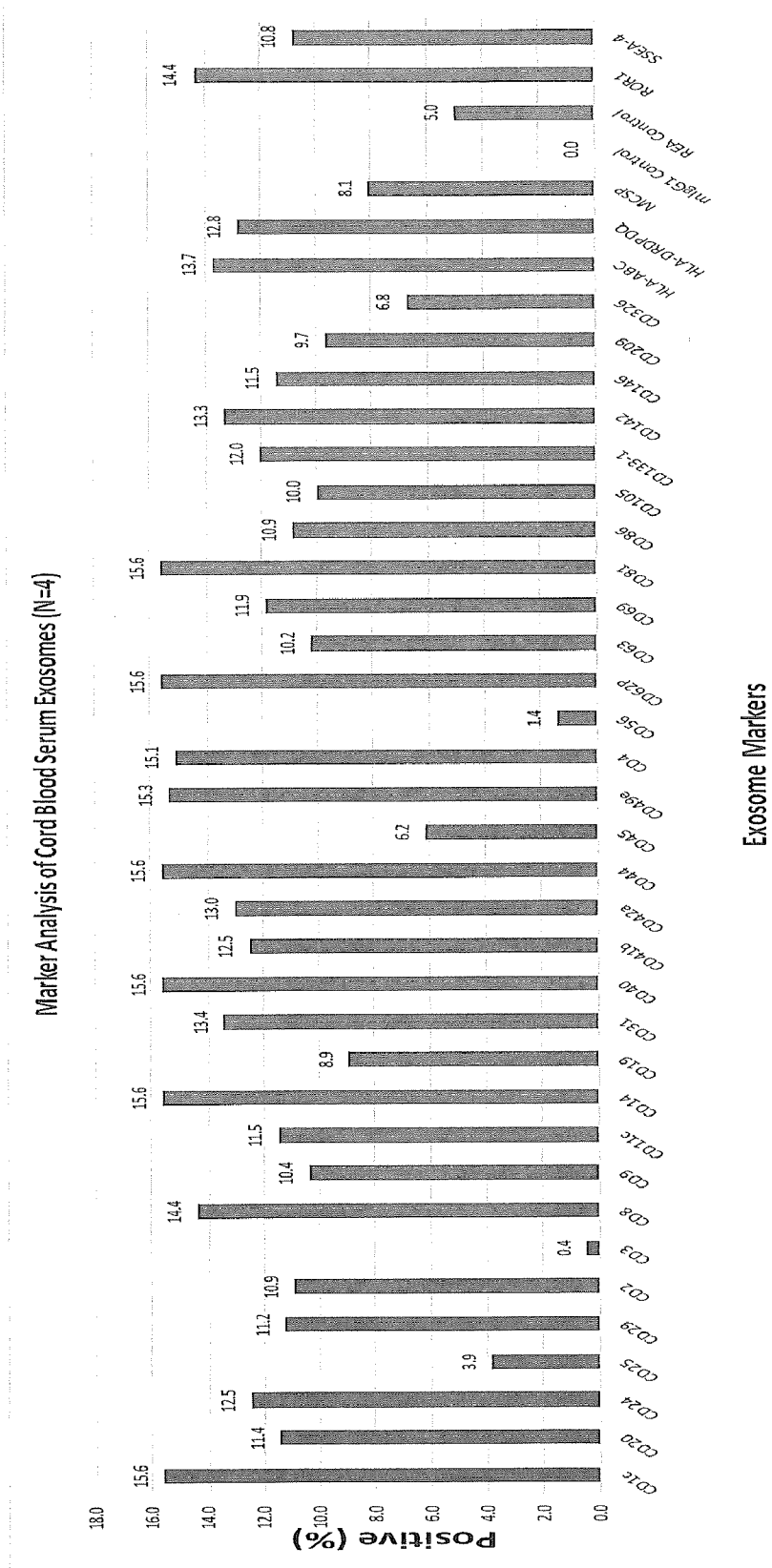

Marker Analysis of Cord Blood Serum Exosomes (N=4)
Positive (%)
Exosome Markers
CD1c
CD20
CD24
CD25
CD29
CD2
CD3
CD8
CD9
CD11c
CD14
CD19
CD31
CD40
CD41b
CD42a
CD44
CD45
CD49e
CD4
CD56
CD62P
CD63
CD69
CD81
CD86
CD105
CD133-1
CD142
CD146
CD209
CD326
HLA-ABC
HLA-DRDPDQ
MCSP
mIgG1 control
REA control
ROR1
SSEA-4

FIG. 3C

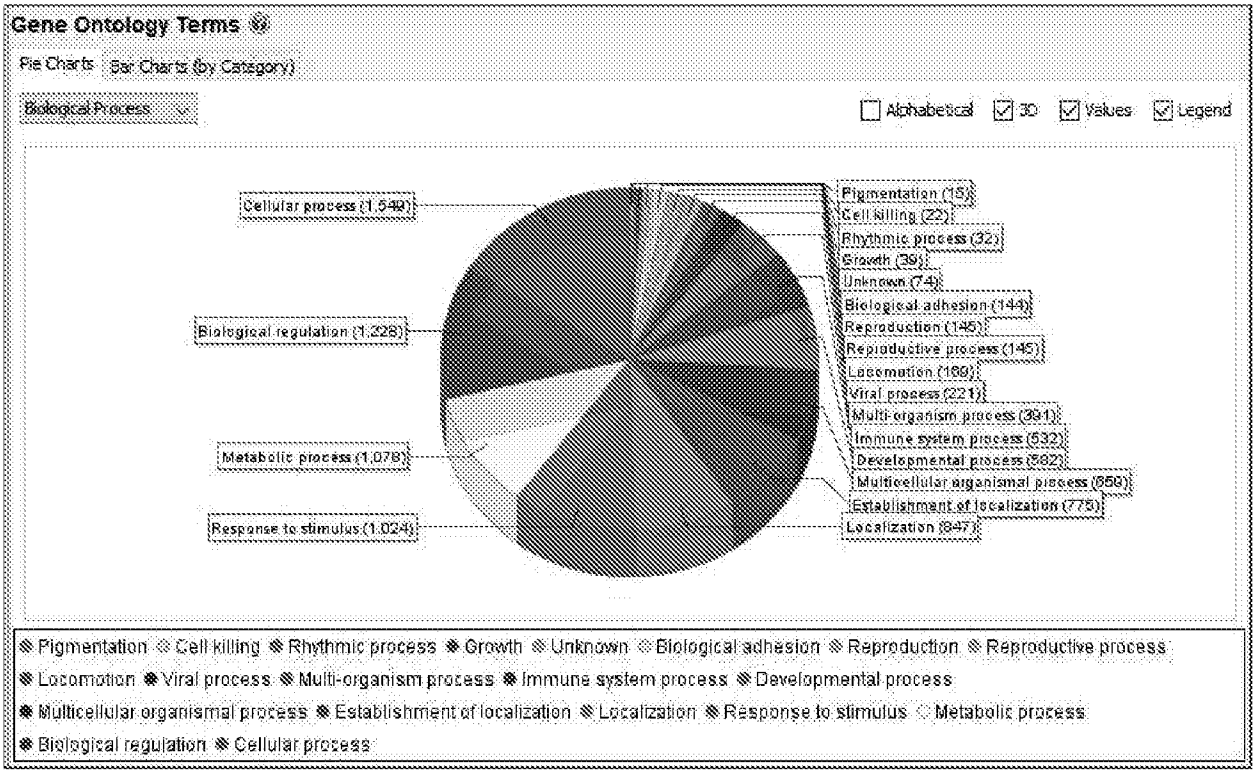

Gene Ontology Terms
Pie Charts
Bar Charts (by Category)
Biological Process
Alphabetical
3D
Values
Legend
Cellular process (1,549)
Biological regulation (1,228)
Metabolic process (1,078)
Response to stimulus (1,024)
Pigmentation (15)
Cell killing (22)
Rhythmic process (32)
Growth (39)
Unknown (74)
Biological adhesion (144)
Reproduction (145)
Reproductive process (145)
Locomotion (169)
Viral process (221)
Multi-organism process (391)
Immune system process (532)
Developmental process (582)
Multicellular organismal process (650)
Establishment of localization (775)
Localization (847)
Pigmentation
Cell killing
Rhythmic process
Growth
Unknown
Biological adhesion
Reproduction
Reproductive process
Locomotion
Viral process
Multi-organism process
Immune system process
Developmental process
Multicellular organismal process
Establishment of localization
Localization
Response to stimulus
Metabolic process
Biological regulation
Cellular process

FIG. 4

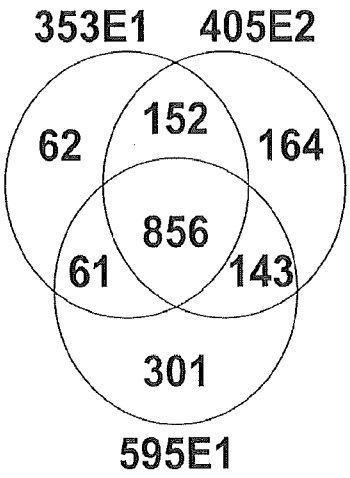


FIG. 5

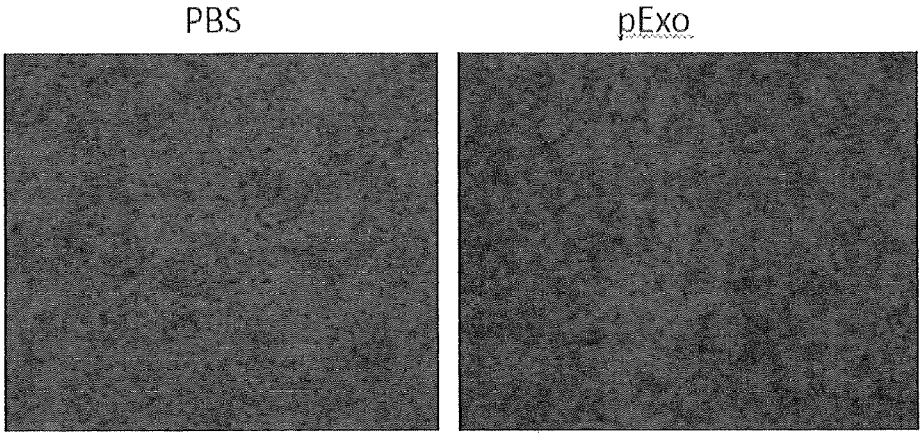


FIG. 6

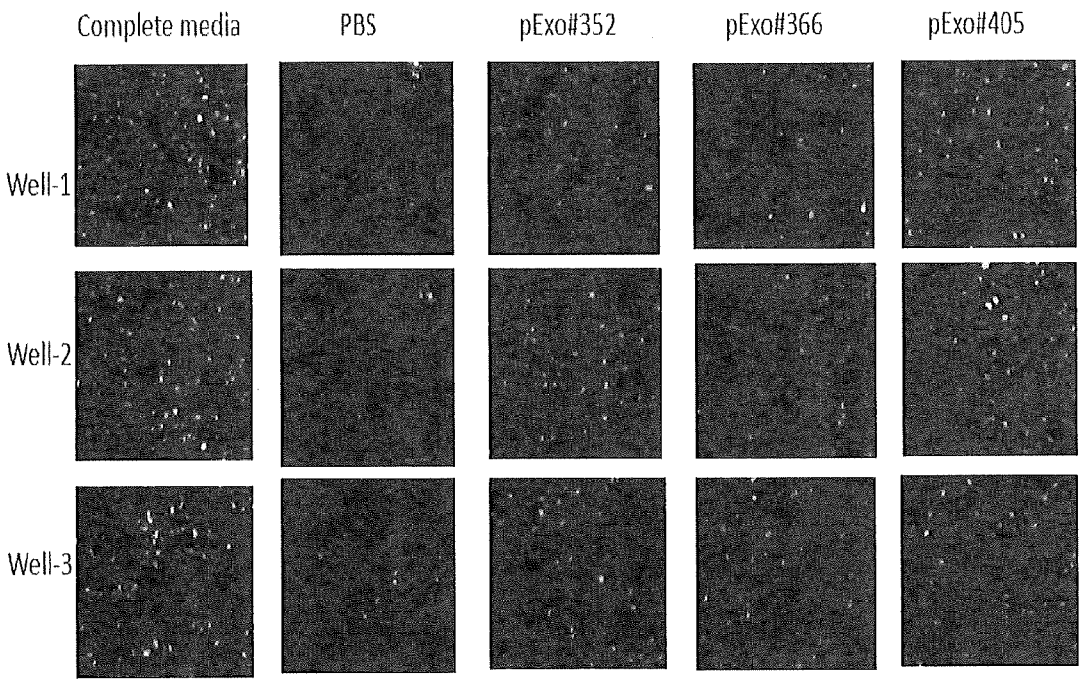
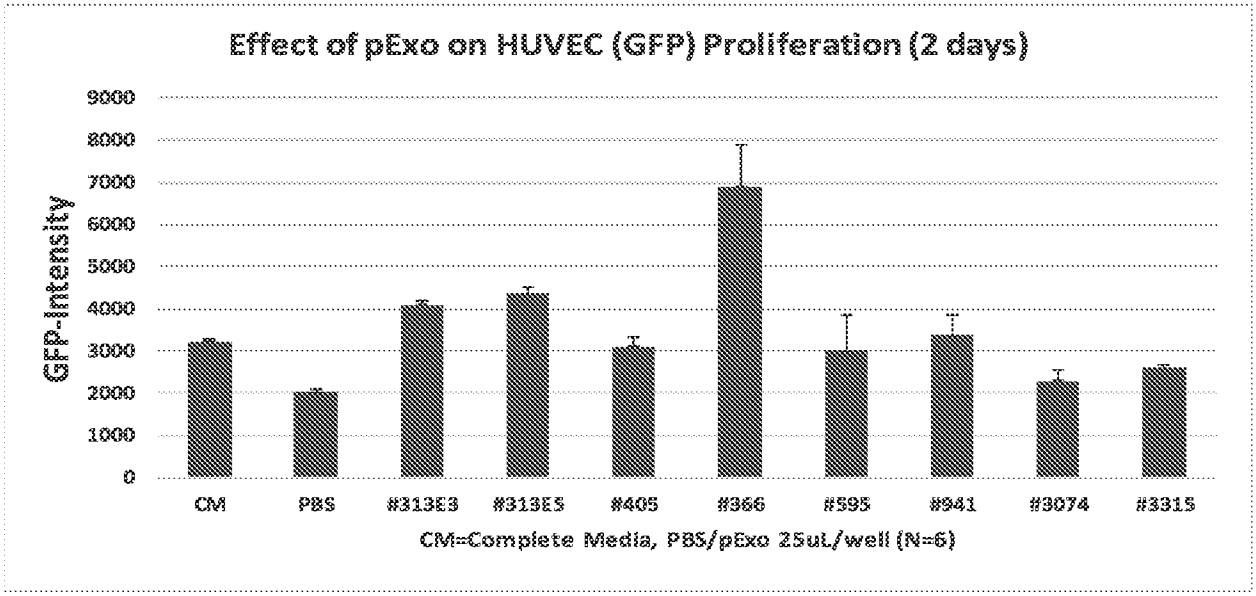

Complete media
PBS
pExo#352
pExo#366
pExo#405
Well-1
Well-2
Well-3

FIG. 7


Effect of pExo on HUVEC (GFP) Proliferation (2 days)
GFP-Intensity
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
CM
PBS
#313E3
#313E5
#405
#366
#595
#941
#3074
#3315
CM=Complete Media, PBS/pExo 25uL/well (N=6)

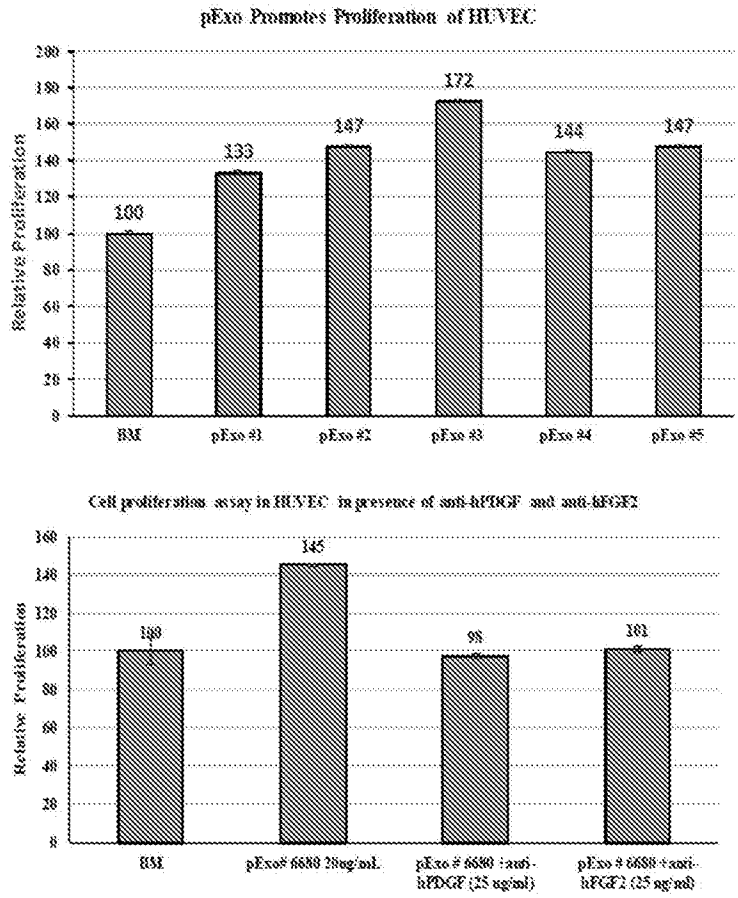

pExo Promotes Proliferation of HUVEC
Relative Proliferation
100
133
147
172
144
147
BM
pExo #1
pExo #2
pExo #3
pExo #4
pExo #5
Cell proliferation assay in HUVEC in presence of anti-hPDGF and anti-hFGF2
Relative Proliferation
100
145
98
101
BM
pExo# 6680 20ug/mL
pExo # 6680 + anti-hPDGF (25 ng/ml)
pExo # 6680 + anti-hFGF2 (25 ng/ml)

FIG. 8

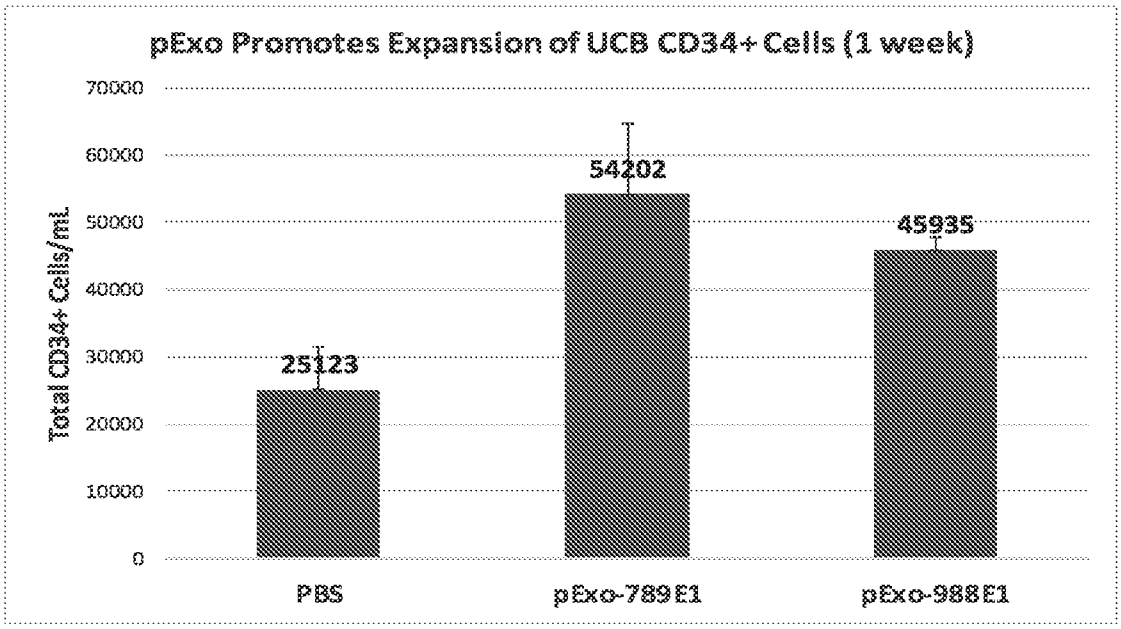

pExo Promotes Expansion of UCB CD34+ Cells (1 week)
Total CD34+ Cells/mL
70000
60000
50000
40000
30000
20000
10000
0
25123
54202
45935
PBS
pExo-789E1
pExo-988E1

FIG. 9

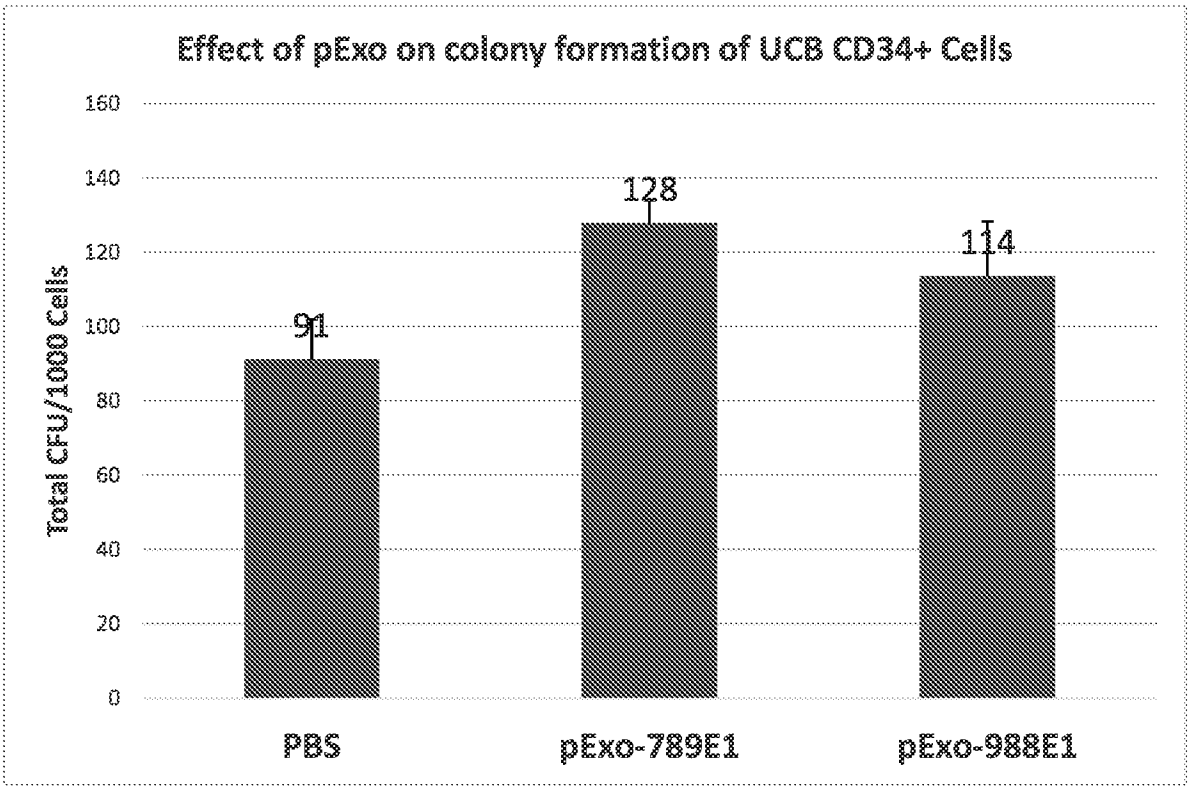

Effect of pExo on colony formation of UCB CD34+ Cells
Total CFU/1000 Cells
160
140
120
100
80
60
40
20
0
91
128
114
PBS
pExo-789E1
pExo-988E1

FIG. 10

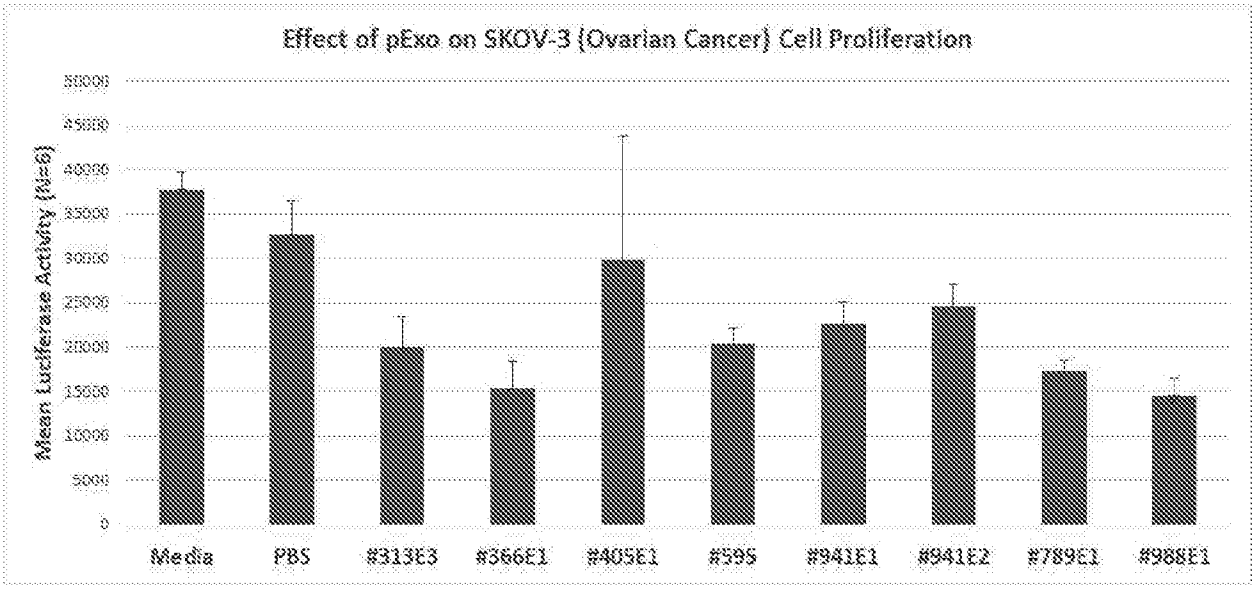

Effect of pExo on SKOV-3 (Ovarian Cancer) Cell Proliferation
Mean Luciferase Activity (N=6)
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
Media
PBS
#313E3
#366E1
#405E1
#595
#941E1
#941E2
#789E1
#988E1

FIG. 11

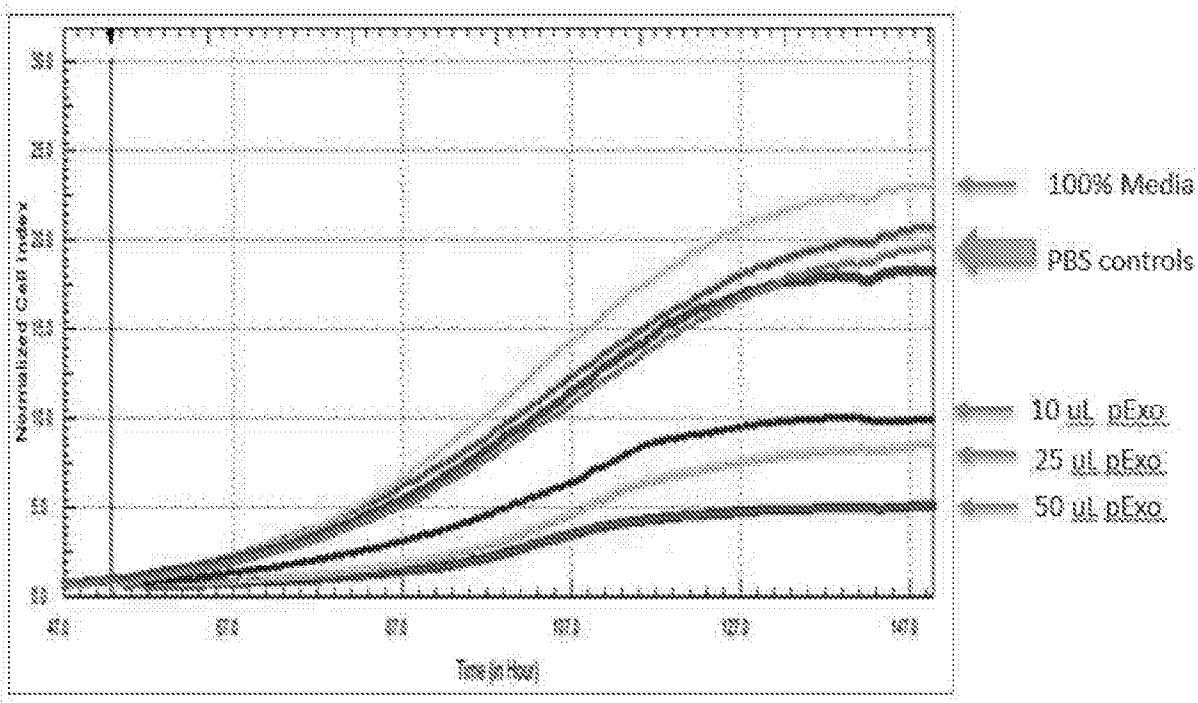


FIG. 12

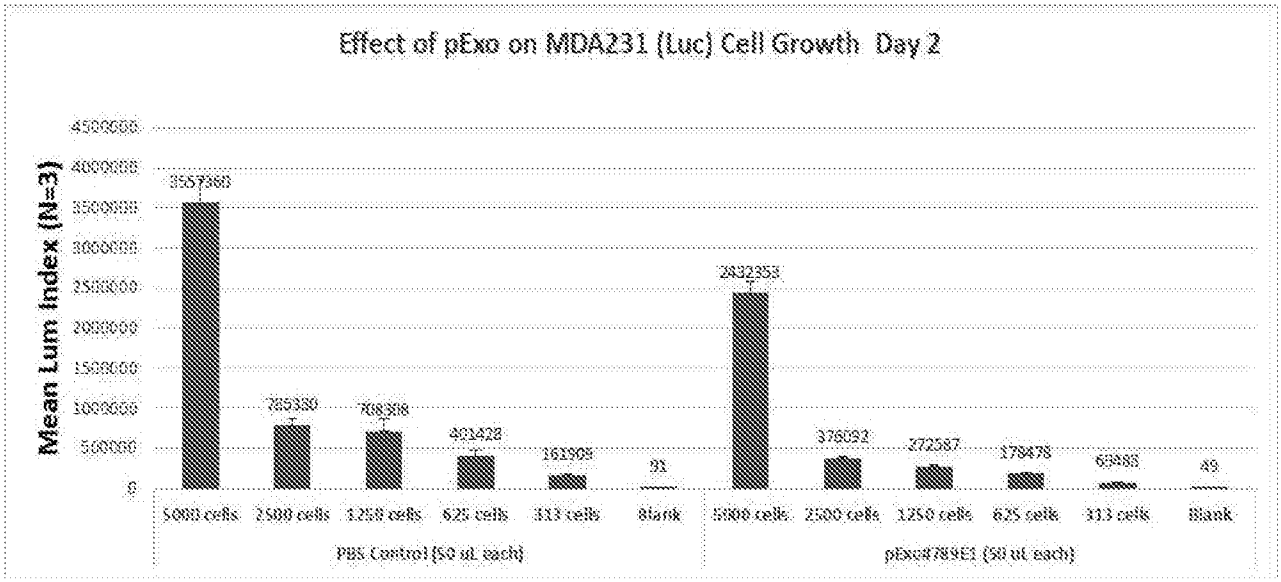

Effect of pExo on MDA231 (Luc) Cell Growth Day 2
Mean Lum Index (N=3)
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
3557360
785330
718308
401428
161905
91
2832353
376092
272587
178478
69885
49
5000 cells
2500 cells
1250 cells
625 cells
313 cells
Blank
5000 cells
2500 cells
1250 cells
625 cells
313 cells
Blank
PBS Control (50 uL each)
pExo#789E1 (50 uL each)

FIG. 13

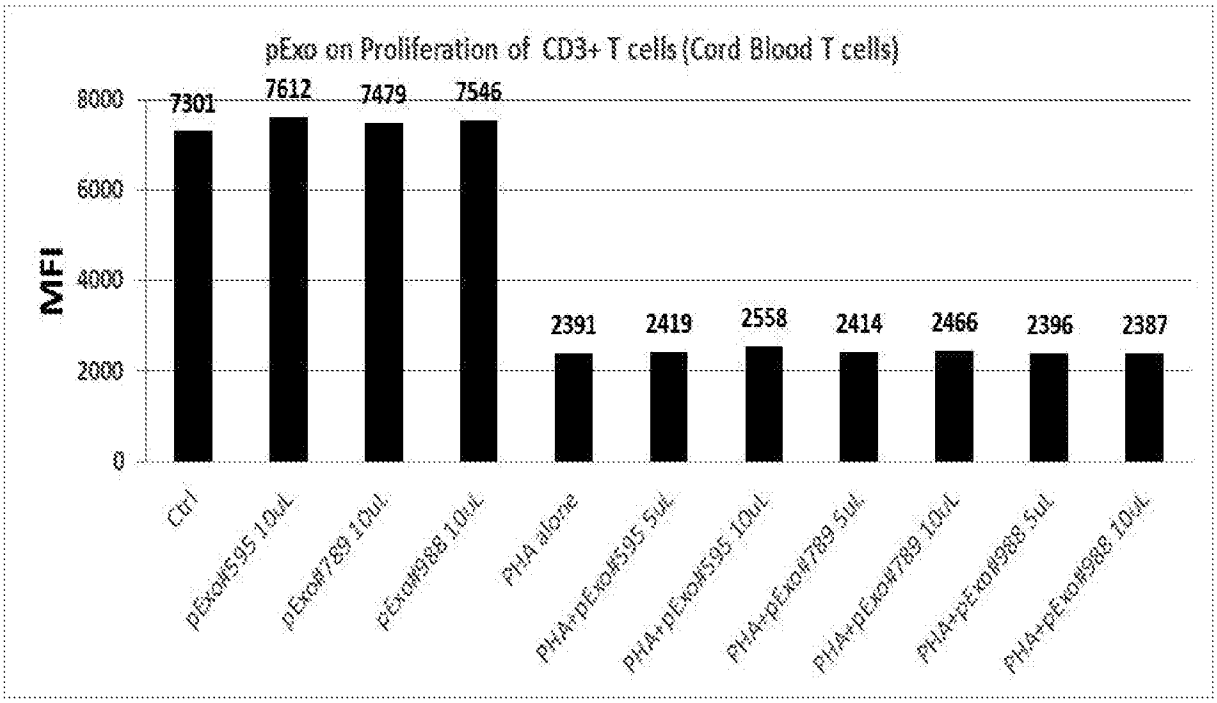

pExo on Proliferation of CD3+ T cells (Cord Blood T cells)
MFI
8000
6000
4000
2000
0
7301
7612
7479
7546
2391
2419
2558
2414
2466
2396
2387
Ctrl
pExo#595 10uL
pExo#789 10uL
pExo#988 10uL
PHA alone
PHA+pExo#595 5uL
PHA+pExo#595 10uL
PHA+pExo#789 5uL
PHA+pExo#789 10uL
PHA+pExo#988 5uL
PHA+pExo#988 10uL

FIG. 14

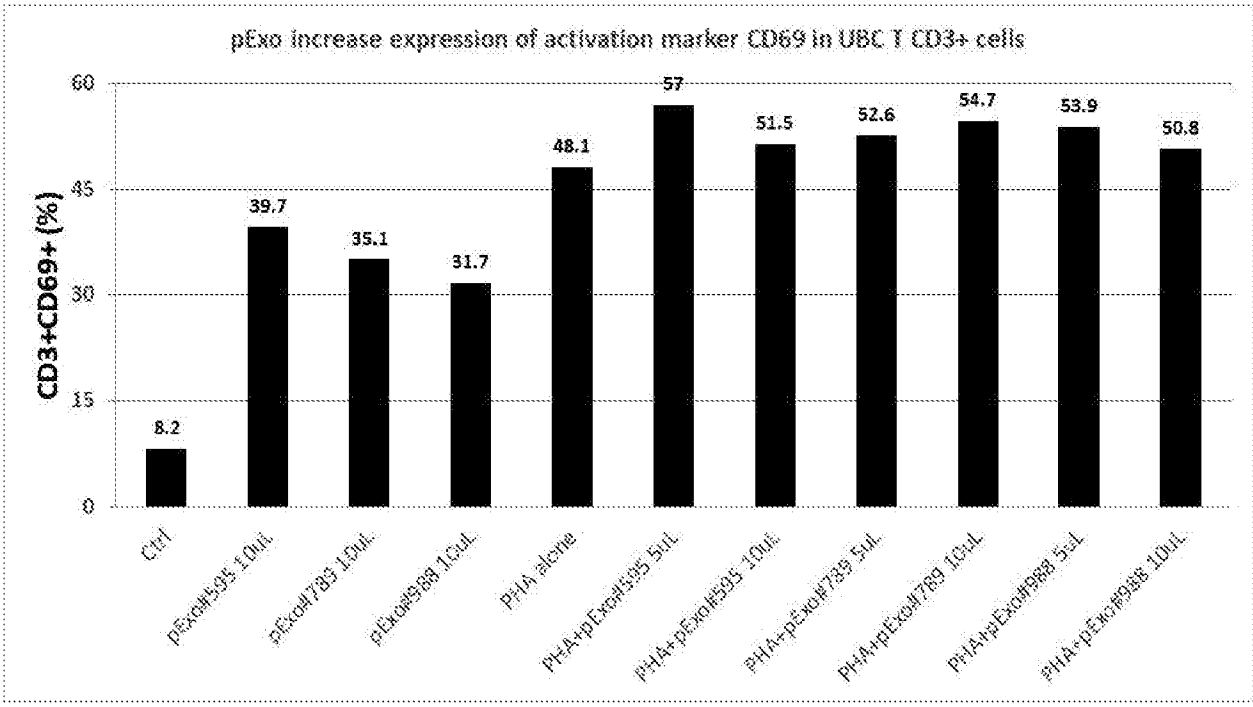

pExo increase expression of activation marker CD69 in UBC T CD3+ cells
CD3+CD69+ (%)
60
45
30
15
0
8.2
39.7
35.1
31.7
48.1
57
51.5
52.6
54.7
53.9
50.8
Ctrl
pExo#595 10uL
pExo#735 10uL
pExo#568 10uL
PHA alone
PHA+pExo#595 5uL
PHA+pExo#595 10uL
PHA+pExo#735 5uL
PHA+pExo#769 10uL
PHA+pExo#988 5uL
PHA+pExo#986 10uL

FIG. 15

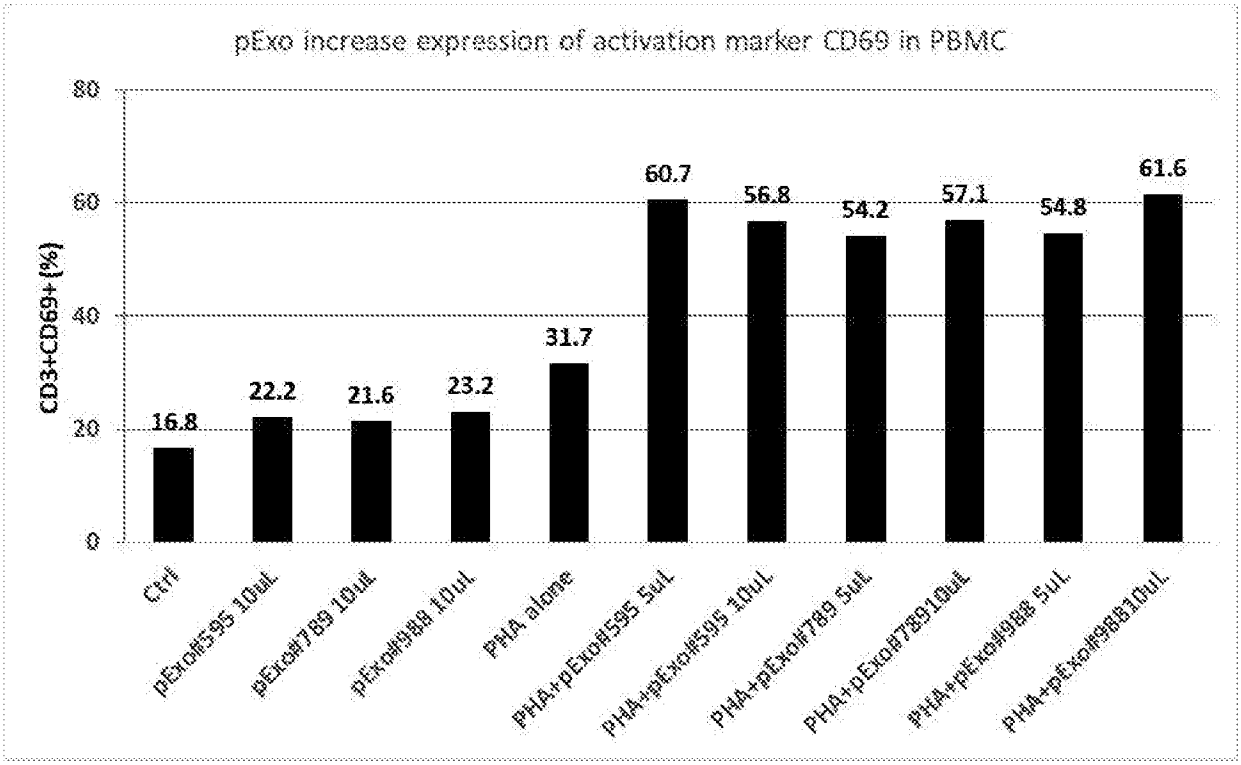

pExo increase expression of activation marker CD69 in PBMC
CD3+CD69+(%)
80
60
40
20
0
16.8
22.2
21.6
23.2
31.7
60.7
56.8
54.2
57.1
54.8
61.6
Ctrl
pExo#595 10uL
pExo#789 10uL
pExo#988 10uL
PHA alone
PHA+pExo#595 5uL
PHA+pExo#595 10uL
PHA+pExo#789 5uL
PHA+pExo#789 10uL
PHA+pExo#988 5uL
PHA+pExo#988 10uL

FIG. 16

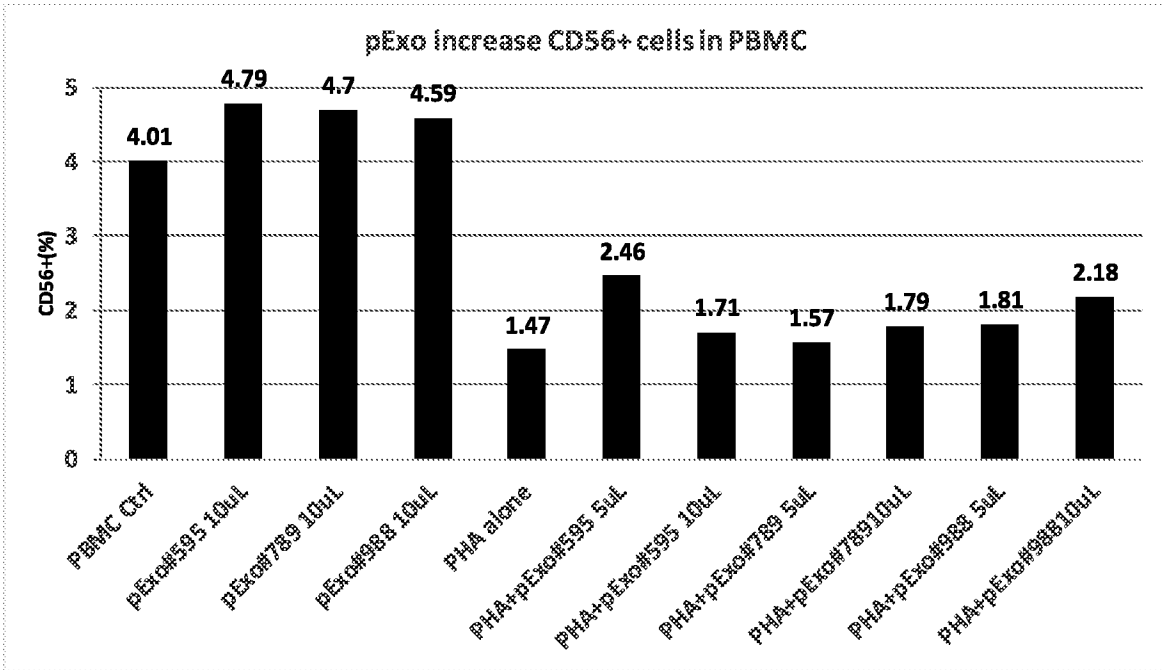
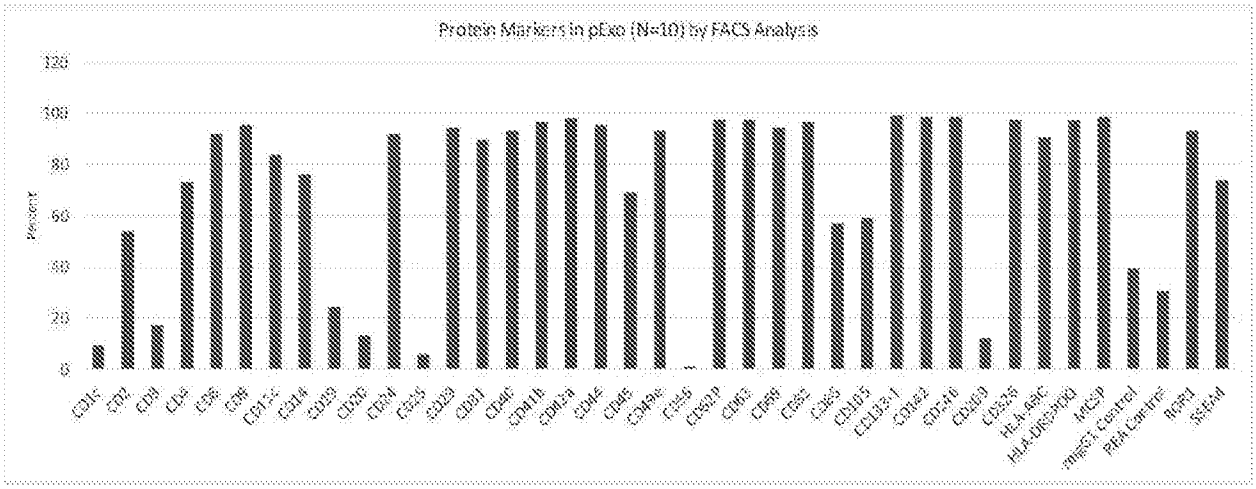

pExo increase CD56+ cells in PBMC
CD56+(%)
0
1
2
3
4
5
4.01
4.79
4.7
4.59
1.47
2.46
1.71
1.57
1.79
1.81
2.18
PBMC Ctrl
pExo#595 10uL
pExo#789 10uL
pExo#988 10uL
PHA alone
PHA+pExo#595 5uL
PHA+pExo#595 10uL
PHA+pExo#789 5uL
PHA+pExo#789 10uL
PHA+pExo#988 5uL
PHA+pExo#988 10uL

FIG. 17


Protein Markers in pExo (N=10) by FACS Analysis
Percent
120
100
80
60
40
20
0
CD1c
CD2
CD3
CD4
CD5
CD8
CD11c
CD14
CD19
CD20
CD24
CD25
CD29
CD31
CD41b
CD42a
CD44
CD45
CD49e
CD56
CD62P
CD63
CD69
CD81
CD86
CD105
CD133.1
CD142
CD146
CD209
CD326
HLA-ABC
HLA-DR,DP,DQ
MCSP
mIgG1 Control
RSA Control
ROR1
SSEA4

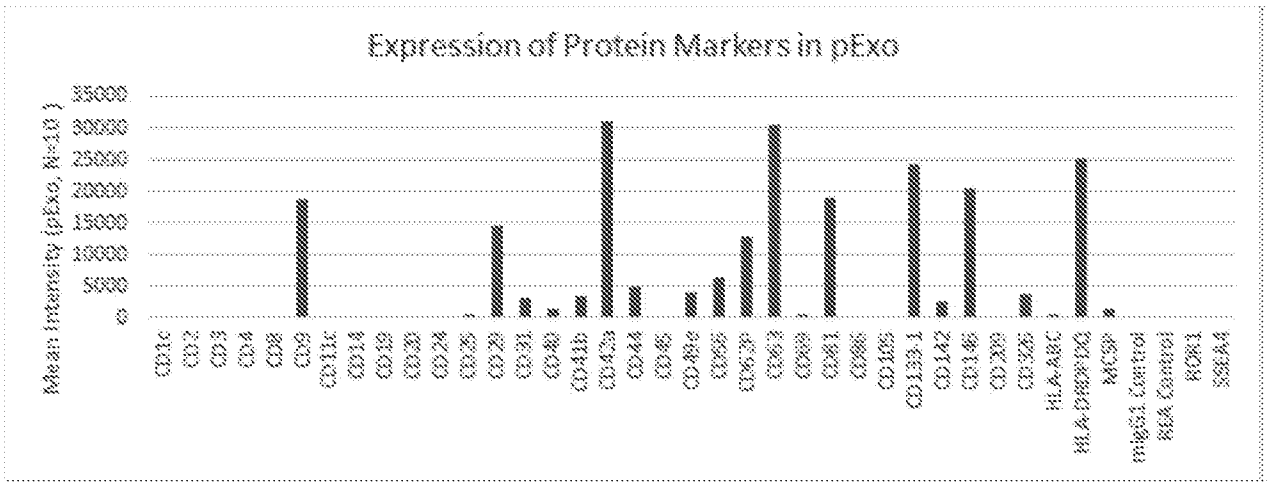
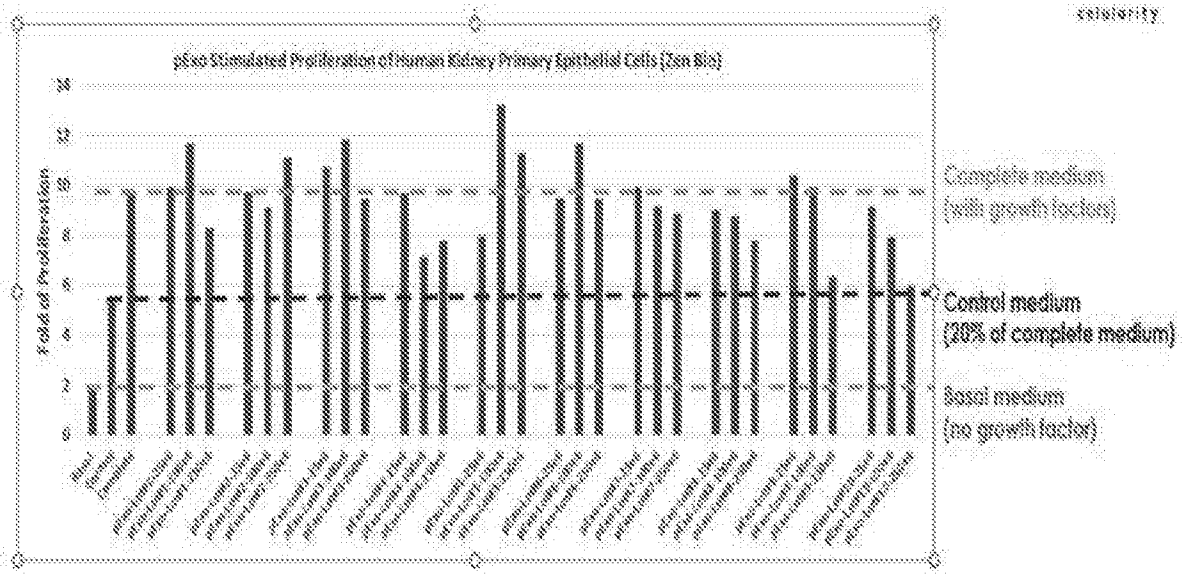

Expression of Protein Markers in pExo
Mean Intensity (pExo, N=10)
35000
30000
25000
20000
15000
10000
5000
0
CD1c
CD2
CD3
CD4
CD5
CD9
CD11c
CD14
CD19
CD20
CD24
CD25
CD29
CD31
CD41b
CD42a
CD44
CD45
CD49e
CD56
CD62P
CD63
CD69
CD81
CD86
CD105
CD133.1
CD142
CD146
CD209
CD326
HLA-ABC
HLA-DR,DP,DQ
MCSP
mIgG1 Control
RSA Control
ROR1
SSEA4

FIG. 18


pExo Stimulated Proliferation of Human Kidney Primary Epithelial Cells (Zen Bio)
Fold of Proliferation
Complete medium
(with growth factors)
Control medium
(20% of complete medium)
Basal medium
(no growth factor)

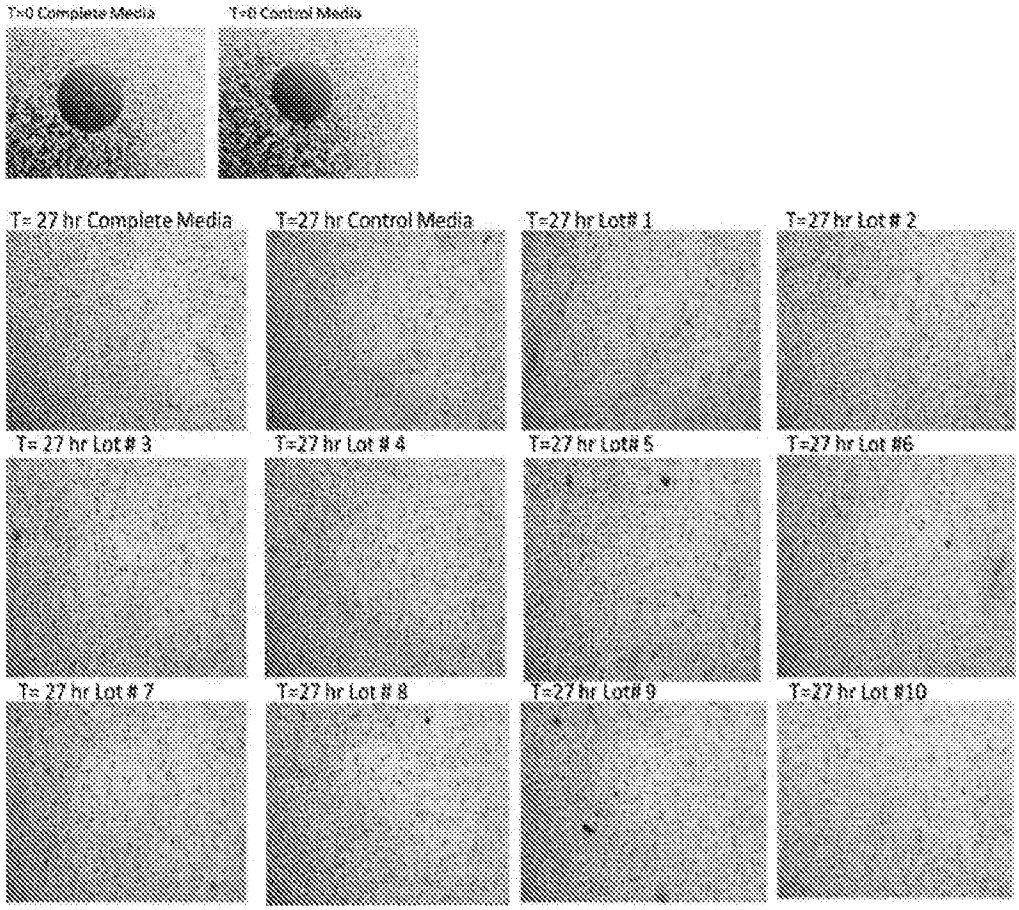

T=0 Complete Media
T=0 Control Media
T= 27 hr Complete Media
T=27 hr Control Media
T=27 hr Lot# 1
T=27 hr Lot # 2
T= 27 hr Lot # 3
T=27 hr Lot # 4
T=27 hr Lot# 5
T=27 hr Lot #6
T= 27 hr Lot # 7
T=27 hr Lot # 8
T=27 hr Lot# 9
T=27 hr Lot #10

FIG. 19

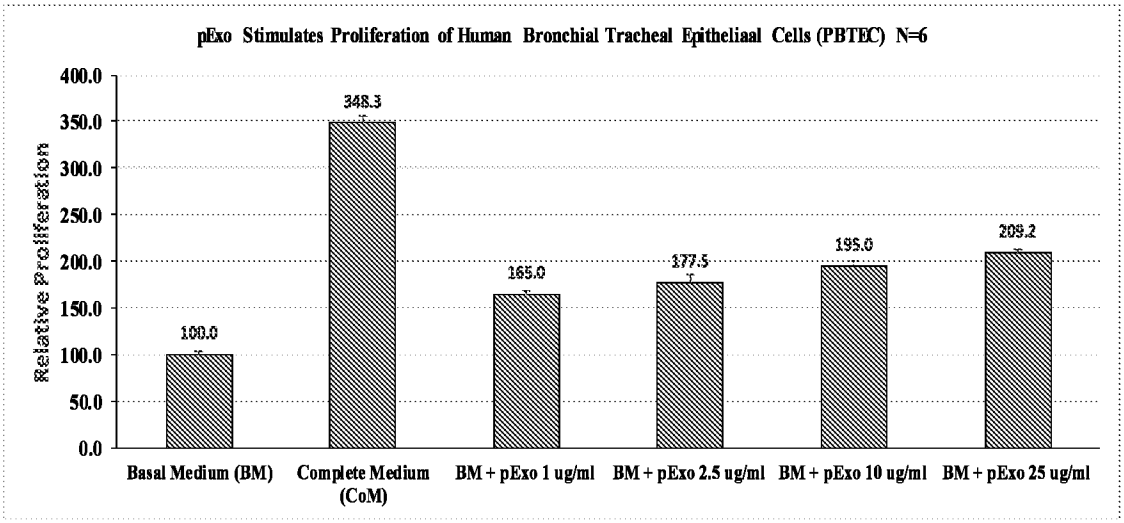
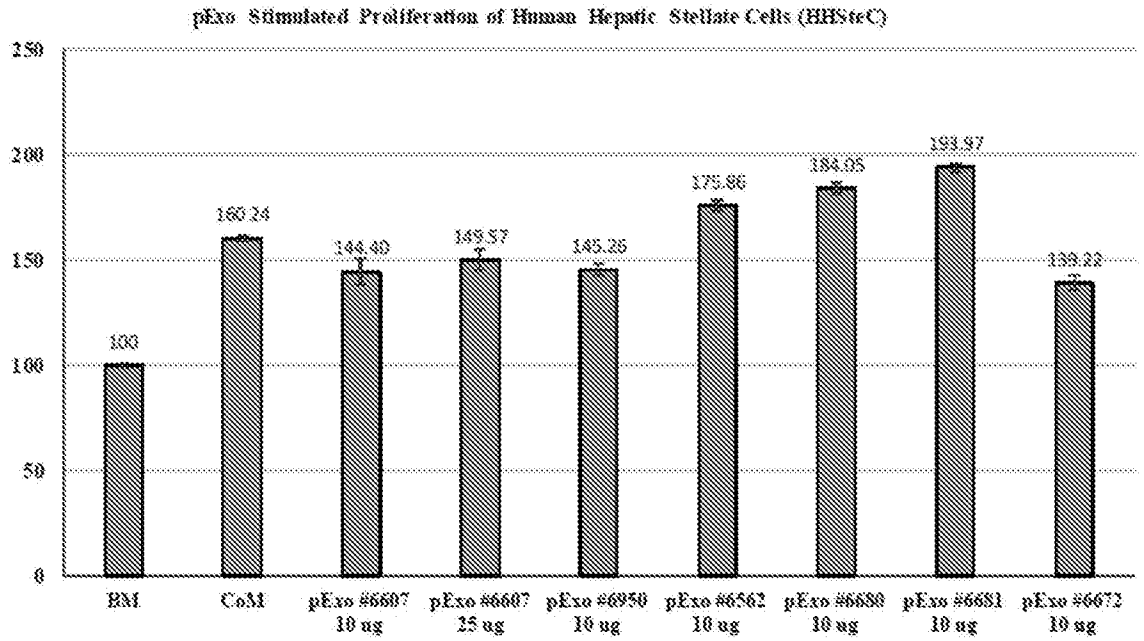

pExo Stimulates Proliferation of Human Bronchial Tracheal Epitheliaal Cells (PBTEC) N=6
Relative Proliferation
400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
100.0
348.3
165.0
177.5
195.0
209.2
Basal Medium (BM)
Complete Medium (CoM)
BM + pExo 1 ug/ml
BM + pExo 2.5 ug/ml
BM + pExo 10 ug/ml
BM + pExo 25 ug/ml

FIG. 20


pExo Stimulated Proliferation of Human Hepatic Stellate Cells (HHSteC)
250
200
150
100
50
0
100
160.24
144.40
149.57
145.26
175.86
184.05
193.97
139.22
BM
CoM
pExo #6607 10 ug
pExo #6607 25 ug
pExo #6950 10 ug
pExo #6562 10 ug
pExo #6680 10 ug
pExo #6681 10 ug
pExo #6672 10 ug

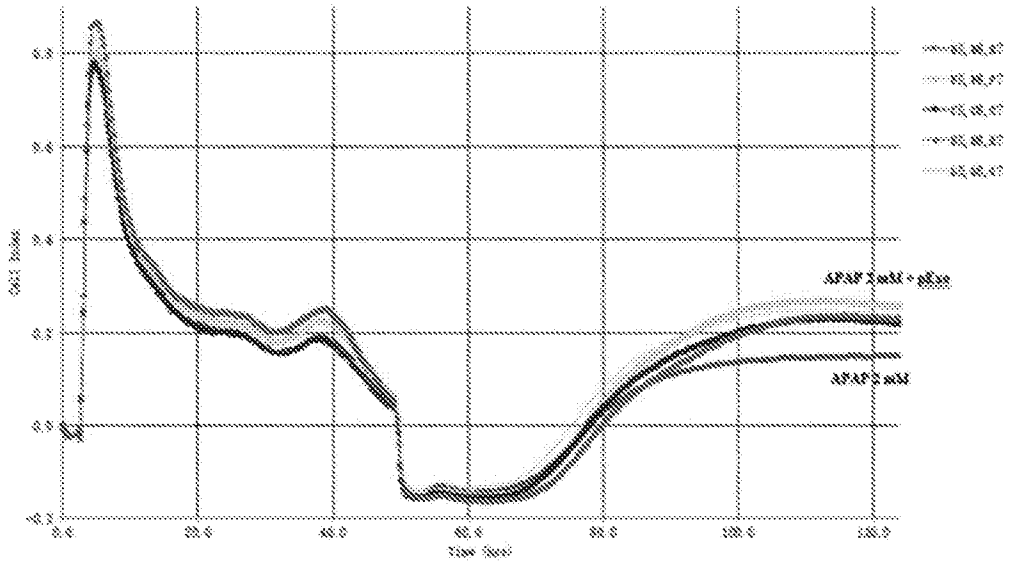


FIG. 21

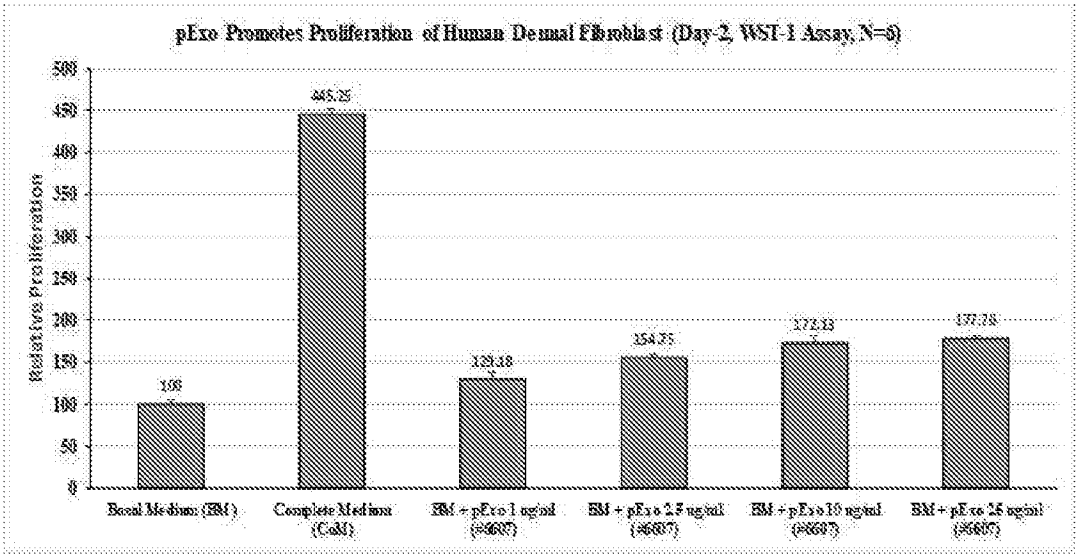
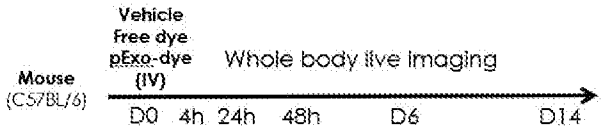
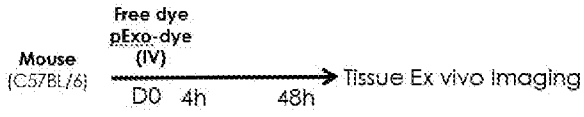

pExo Promotes Proliferation of Human Dermal Fibroblast (Day-2, WST-1 Assay, N=8)
Relative Proliferation
500
450
400
350
300
250
200
150
100
50
0
445.25
129.18
154.75
272.13
177.78
Basal Medium (BM)
Complete Medium (CoM)
BM + pExo 1 ug/ml (#6607)
BM + pExo 2.5 ug/ml (#6607)
BM + pExo 10 ug/ml (#6607)
BM + pExo 25 ug/ml (#6607)

FIG. 22



Vehicle	NA	D0	1	D14
Free Dye	NA	D0	1	D14
Exosome-Dye	300μg	D0	3	D14



Free Dye	NA	D0	1+1	4h, 48h
Exosome-Dye	300μg	D0	3+3	4h, 48h

FIG. 23

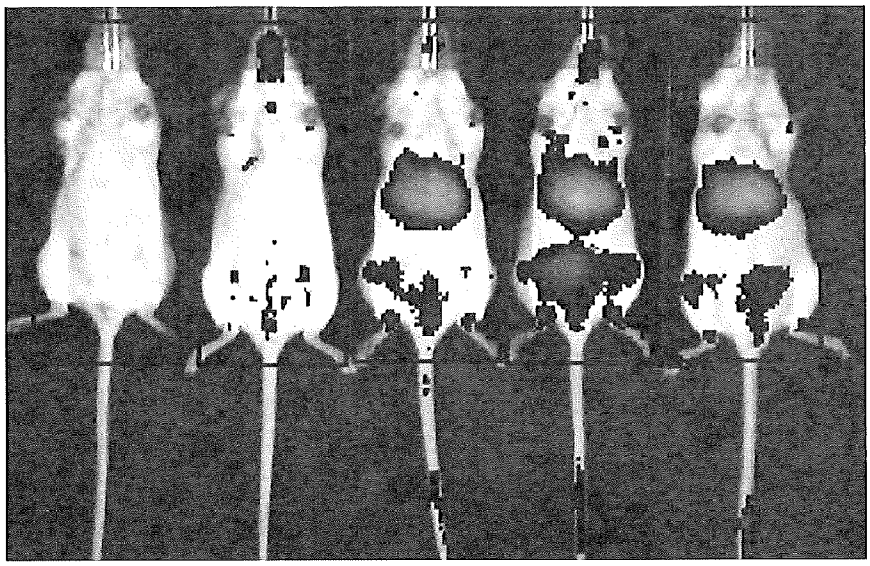


FIG. 24

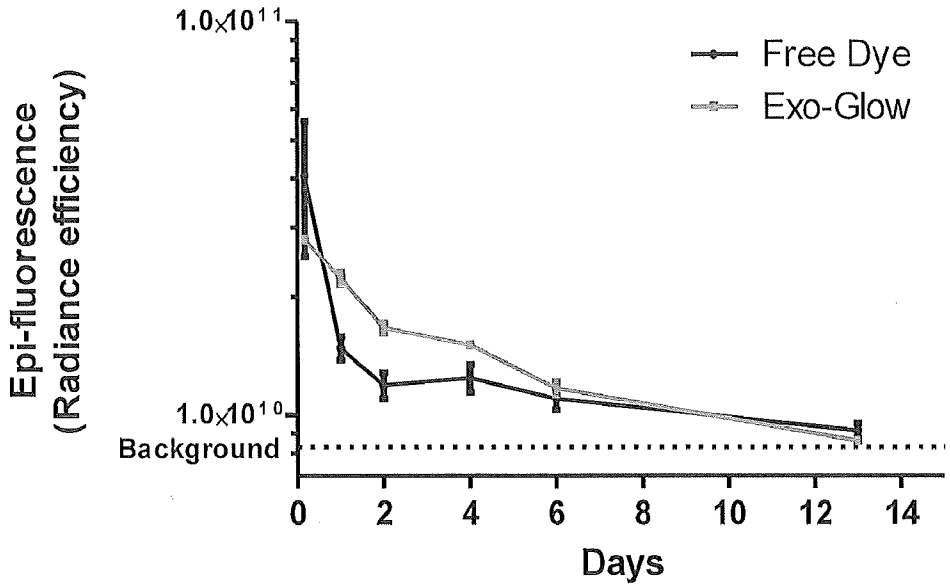
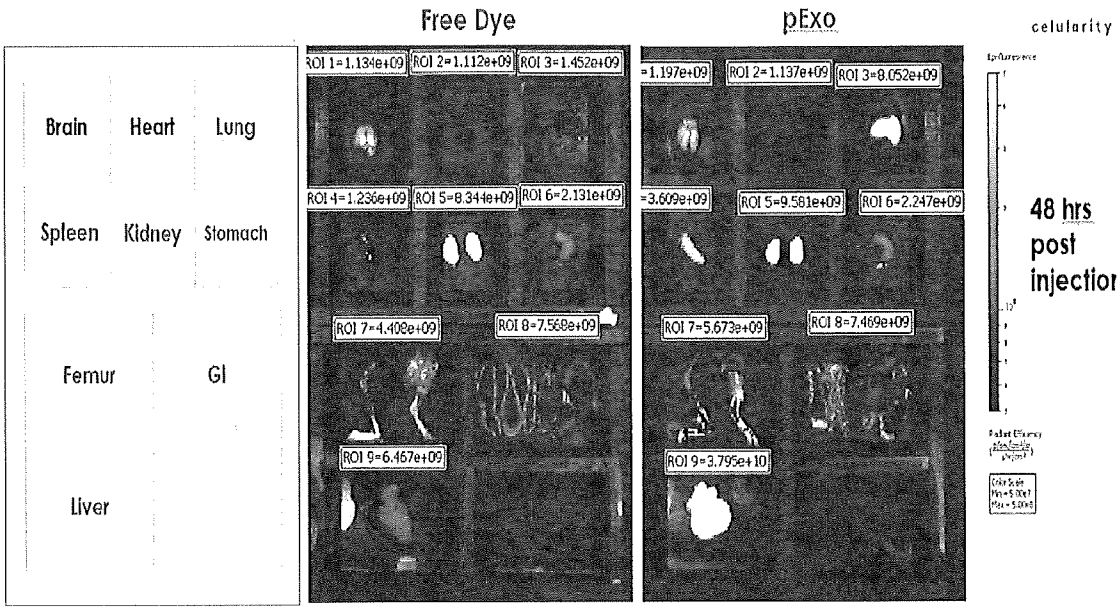
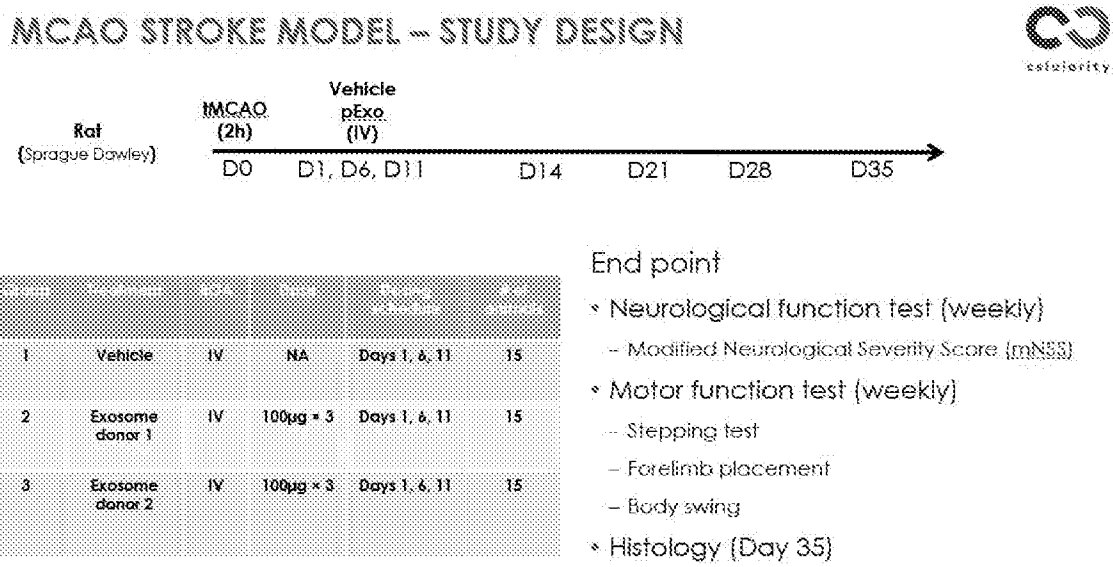

1.0x10^11
1.0x10^10
Background
Epi-fluorescence
(Radiance efficiency)
Free Dye
Exo-Glow
0
2
4
6
8
10
12
14
Days

FIG. 25



- pExo were mainly found in the lung, liver, spleen, kidney, stomach, GI tract and femur (bone marrow)

FIG. 26



1	Vehicle	IV	NA	Days 1, 6, 11	15
2	Exosome donor 1	IV	100µg × 3	Days 1, 6, 11	15
3	Exosome donor 2	IV	100µg × 3	Days 1, 6, 11	15

End point

- Neurological function test (weekly)
  - Modified Neurological Severity Score (mNSS)
- Motor function test (weekly)
  - Stepping test
  - Forelimb placement
  - Body swing
- Histology (Day 35)

FIG. 27

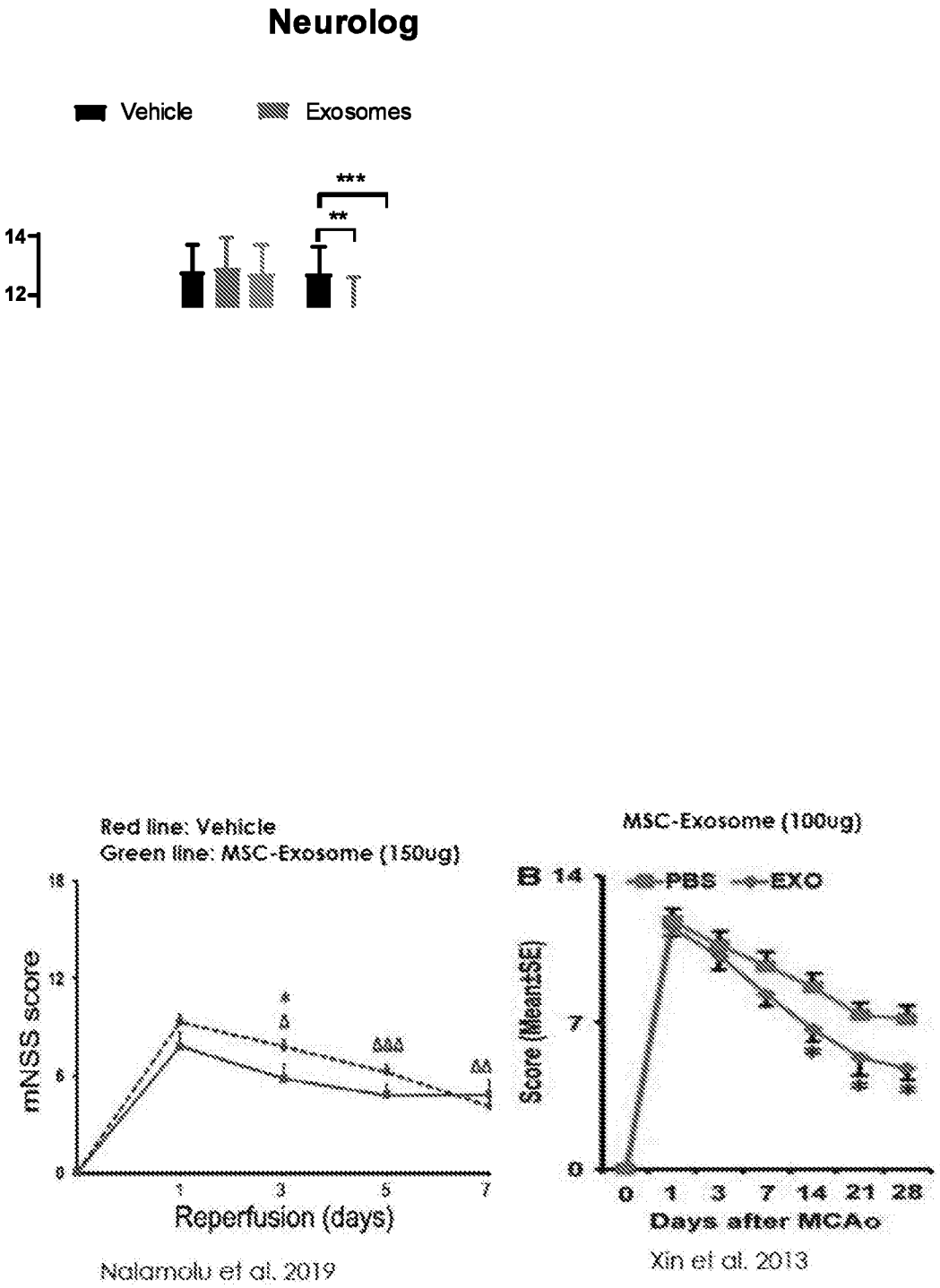

Neurolog
Vehicle
Exosomes
***
**
14
12
Red line: Vehicle
Green line: MSC-Exosome (150ug)
mNSS score
Reperfusion (days)
Nalamolu et al. 2019
MSC-Exosome (100ug)
PBS
EXO
Score (Mean±SE)
Days after MCAo
Xin et al. 2013

FIG. 28

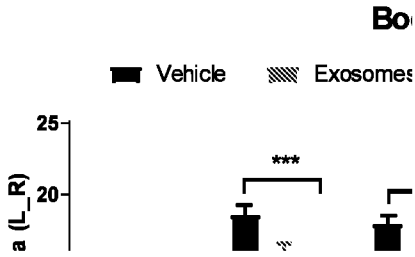


FIG. 29

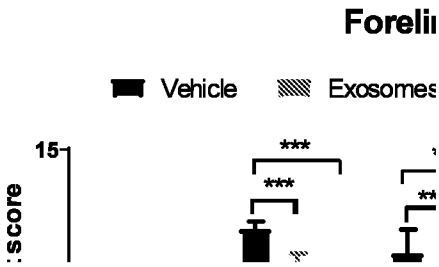


FIG. 30

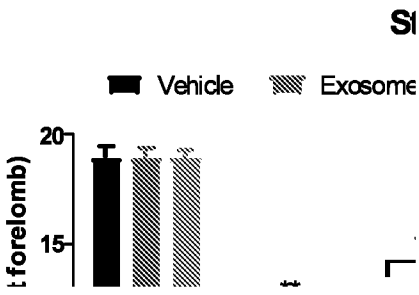


FIG. 31

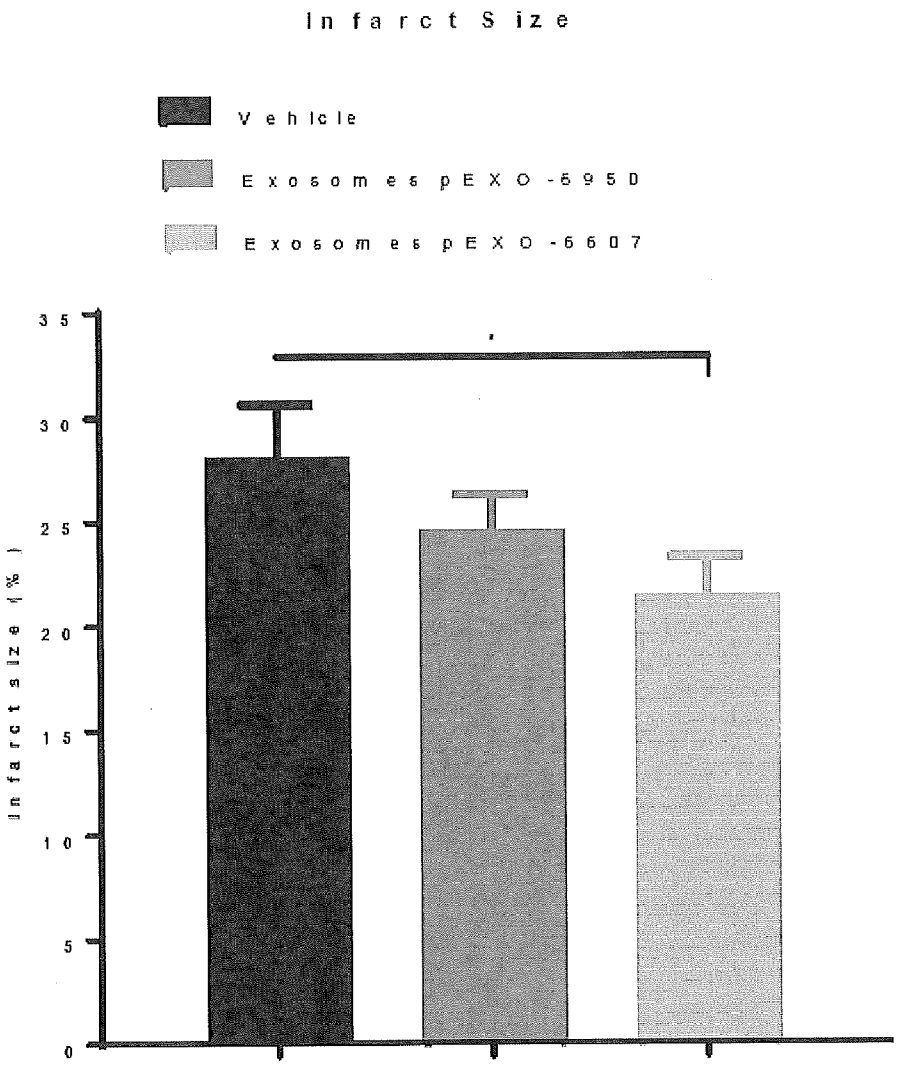

Infarct Size
Vehicle
Exosomes pEXO-5950
Exosomes pEXO-6607
Infarct size (%)
35
30
25
20
15
10
5
0

FIG. 32

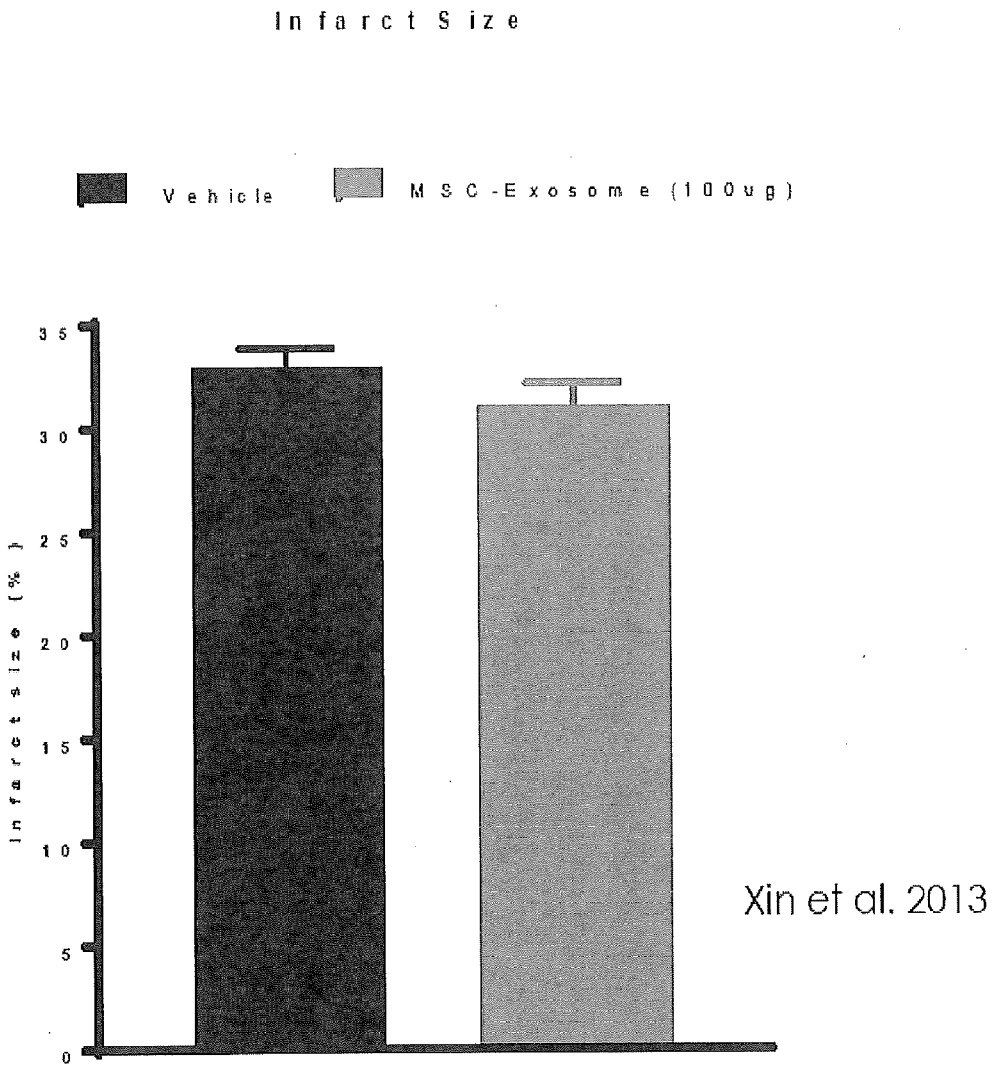

Infarct Size
Vehicle
MSC-Exosome (100ug)
Infarct size (%)
35
30
25
20
15
10
5
0
Xin et al. 2013

FIG. 33

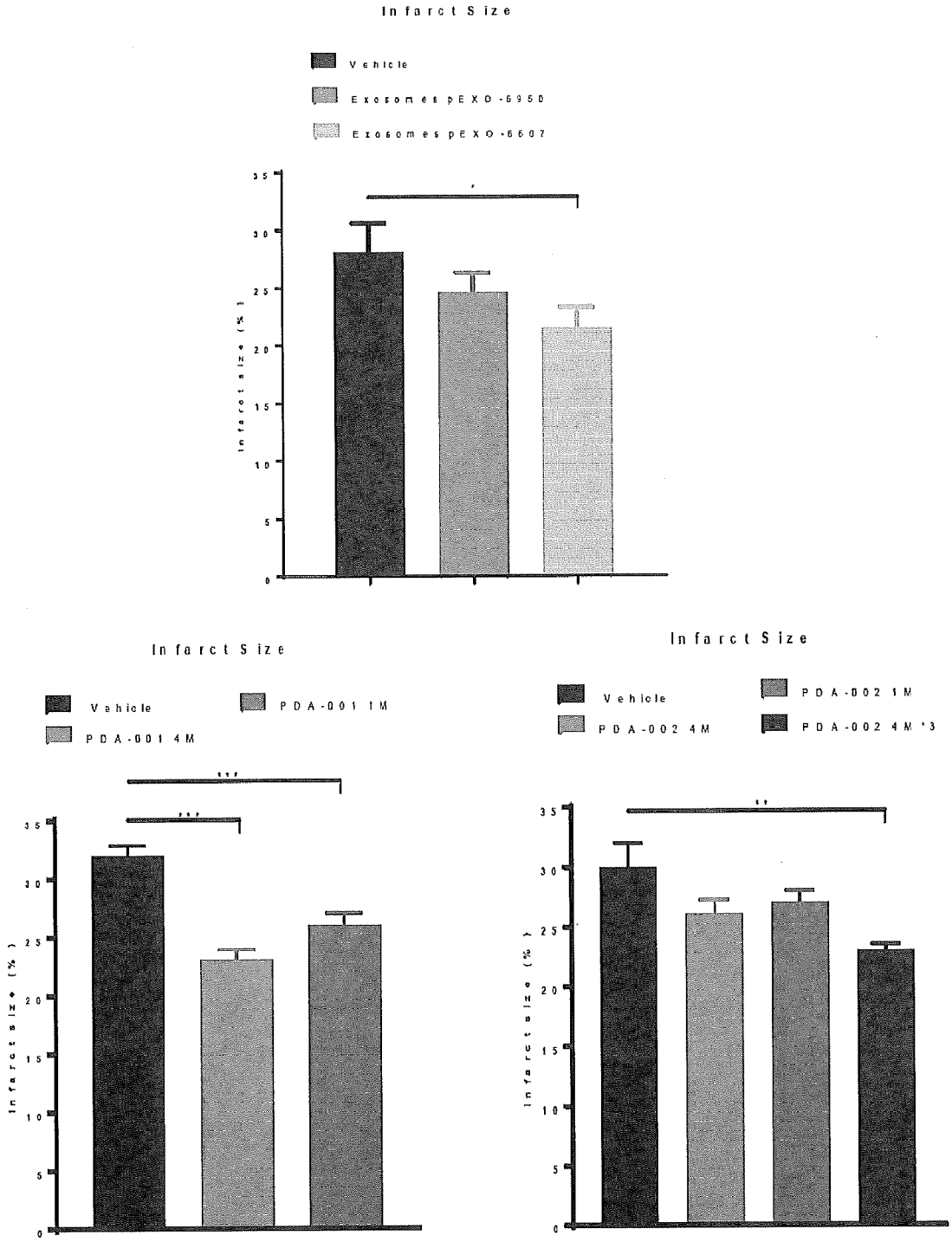

Infarct Size
Vehicle
Exosomes pEXO-6950
Exosomes pEXO-6607
Infarct size (%)
Infarct Size
Vehicle
PDA-001 1M
PDA-001 4M
Infarct Size
Vehicle
PDA-002 1M
PDA-002 4M
PDA-002 4M *3

FIG. 34

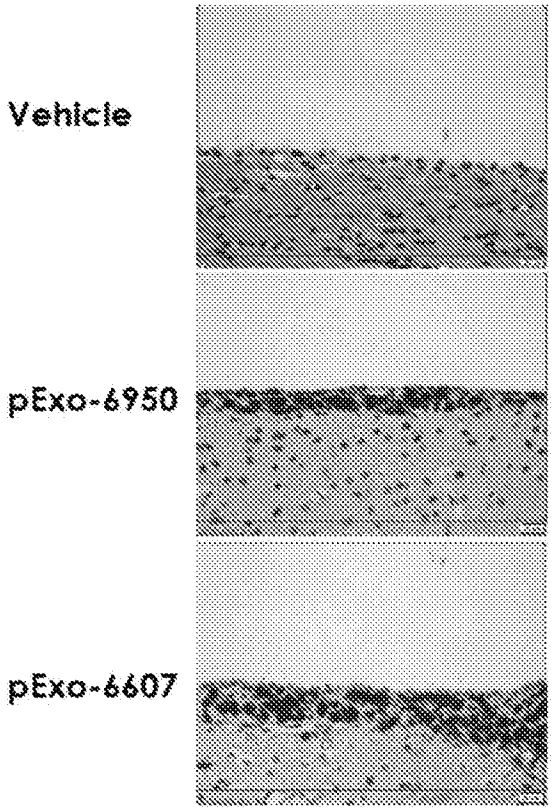


FIG. 35

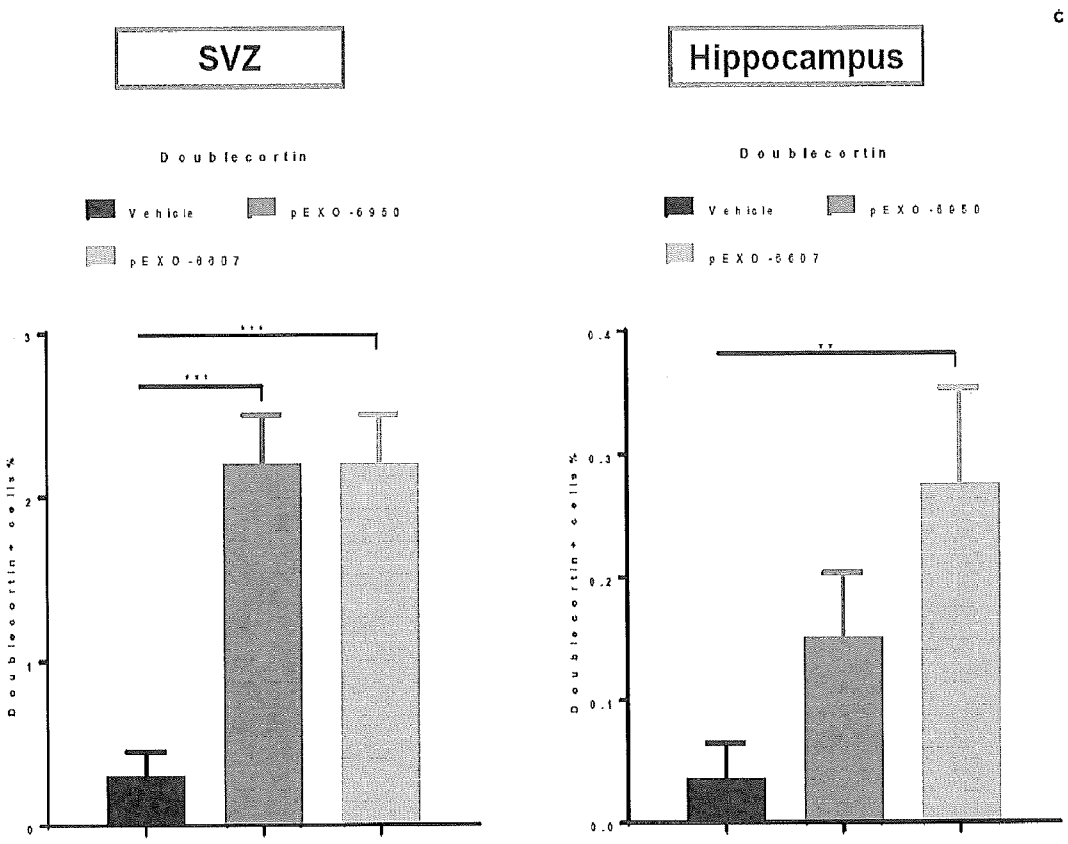
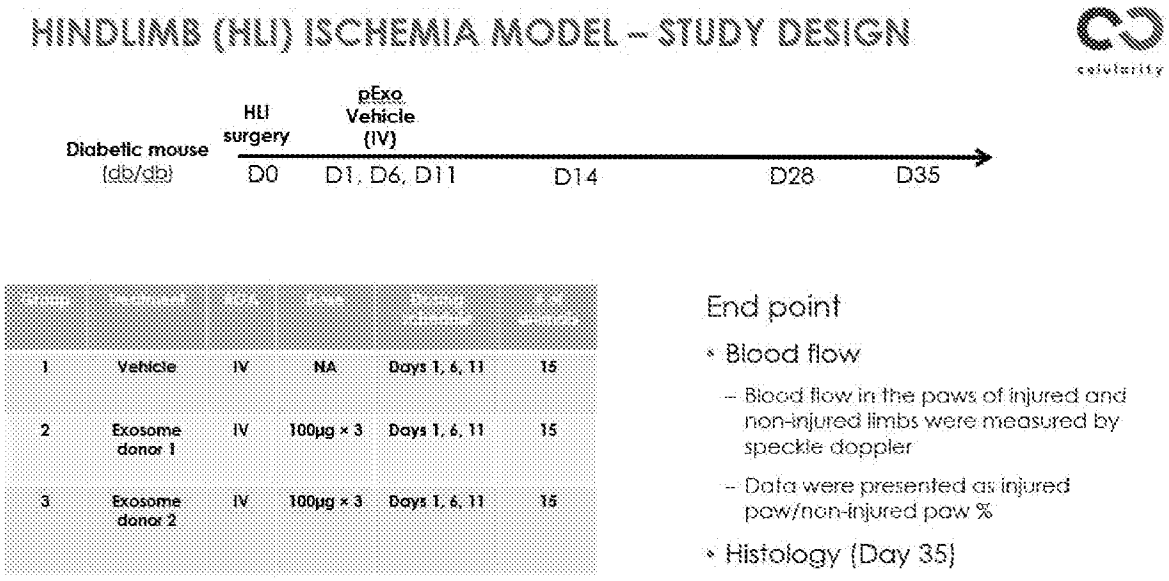

SVZ
Hippocampus
Doublecortin
Vehicle
pEXO-6950
pEXO-8607
Doublecortin+ cells %
***
**

FIG. 36



1	Vehicle	IV	NA	Days 1, 6, 11	15
2	Exosome donor 1	IV	100µg × 3	Days 1, 6, 11	15
3	Exosome donor 2	IV	100µg × 3	Days 1, 6, 11	15

End point

- Blood flow
  - Blood flow in the paws of injured and non-injured limbs were measured by speckle doppler
  - Data were presented as injured paw/non-injured paw %
- Histology (Day 35)

FIG. 37

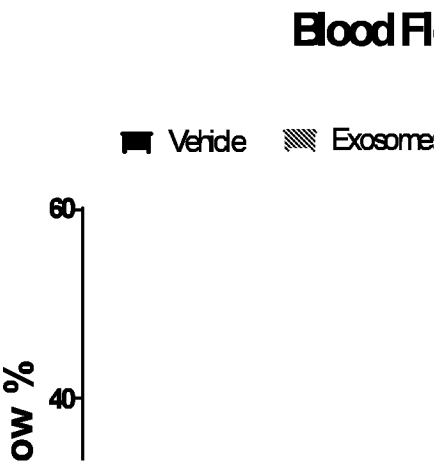


FIG. 38

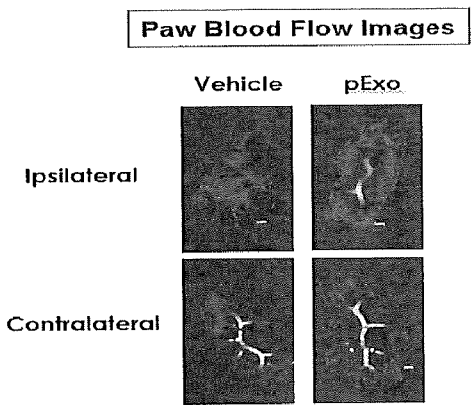

Paw Blood Flow Images
Vehicle
pExo
Ipsilateral
Contralateral

FIG. 39

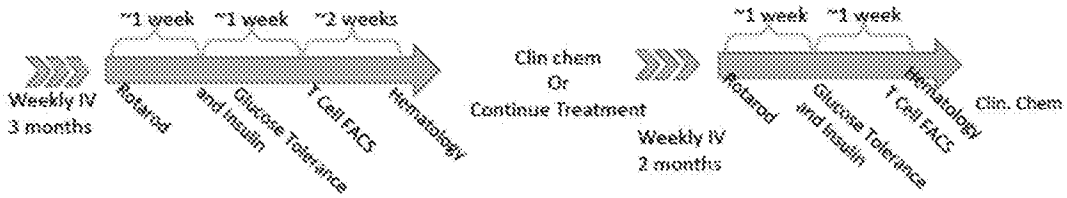

~1 week
~1 week
~2 weeks
Weekly IV
3 months
Rotarod
Glucose Tolerance and Insulin
T Cell FACS
Hematology
Clin chem
Or
Continue Treatment
~1 week
~1 week
Weekly IV
2 months
Rotarod
Glucose Tolerance and Insulin
T Cell FACS
Hematology
Clin. Chem

FIG. 40

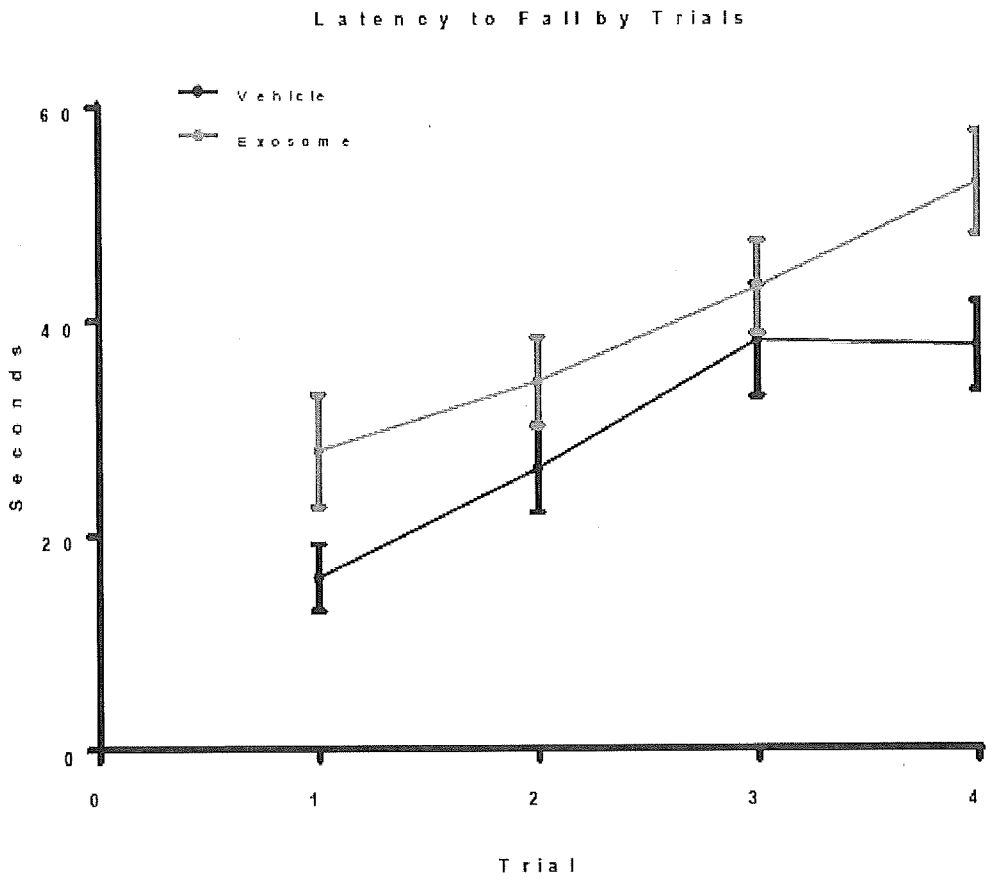

Latency to Fall by Trials
Vehicle
Exosome
60
40
20
0
Seconds
0
1
2
3
4
Trial

FIG. 41

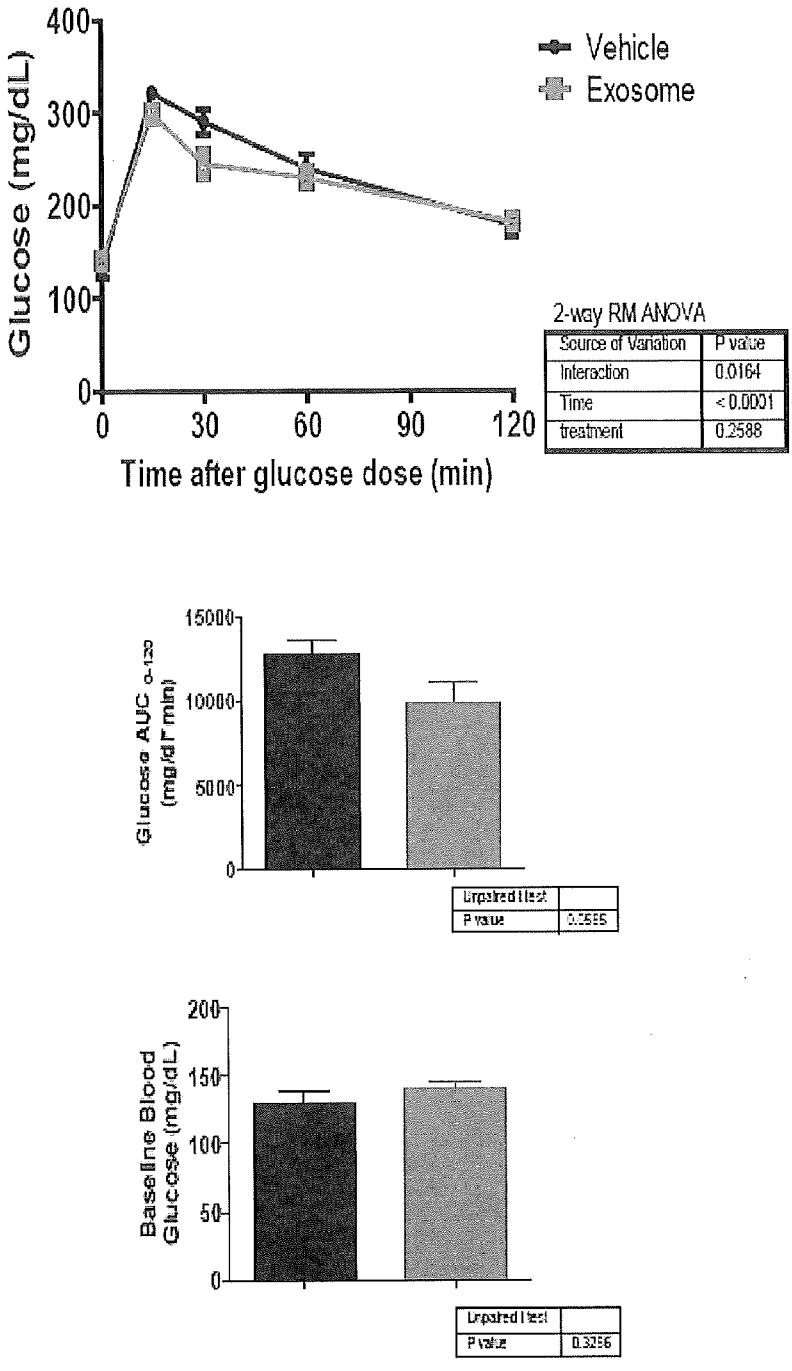

Glucose (mg/dL)
400
300
200
100
0
0
30
60
90
120
Time after glucose dose (min)
Vehicle
Exosome
2-way RM ANOVA
Source of Variation | P value
Interaction | 0.0164
Time | < 0.0001
treatment | 0.2588
Glucose AUC 0-120 (mg/dl*min)
15000
10000
5000
0
Unpaired t test
P value
0.2595
Baseline Blood Glucose (mg/dL)
200
150
100
50
0
Unpaired t test
P value
0.3256

FIG. 42

Group	Treatment	Dosing schedule for Exosomes	# of NCG animals	PBMC cell number and Injection	ROA
1	HuPBMCs +FBS	Day7	6	30M/Day0	IV
2	HuPBMCs+Exosomes	Day7	6	30M/Day0	IV
3	HuPBMCs+Exosomes	Days7, 14, 21	6	30M/Day0	IV

FIG. 43

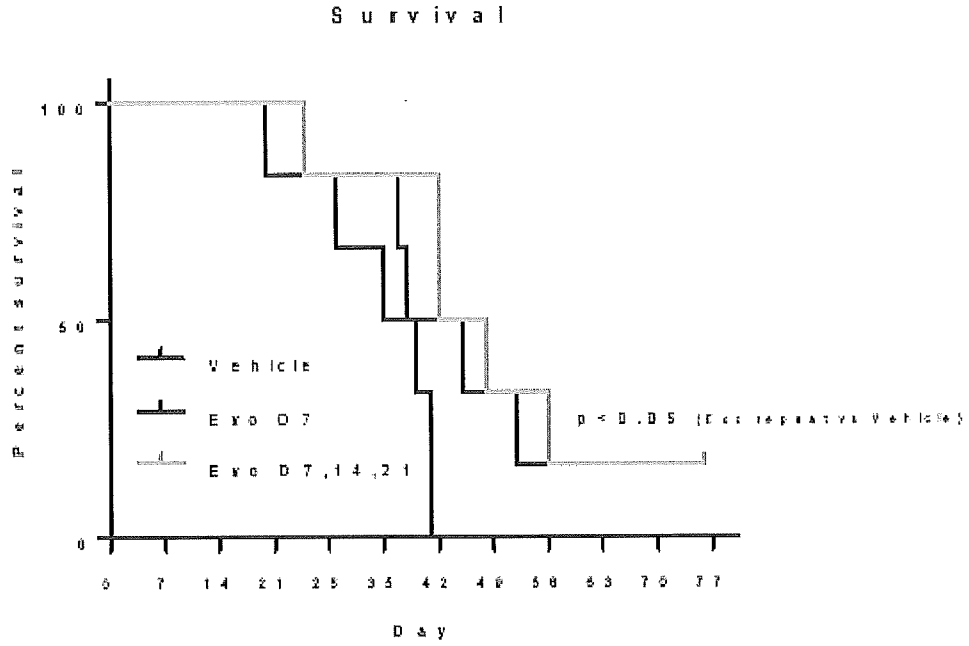

Survival
Percent survival
100
50
0
Vehicle
Exo D7
Exo D7,14,21
p < 0.05
0 7 14 21 28 35 42 49 56 63 70 77
Day

FIG. 44

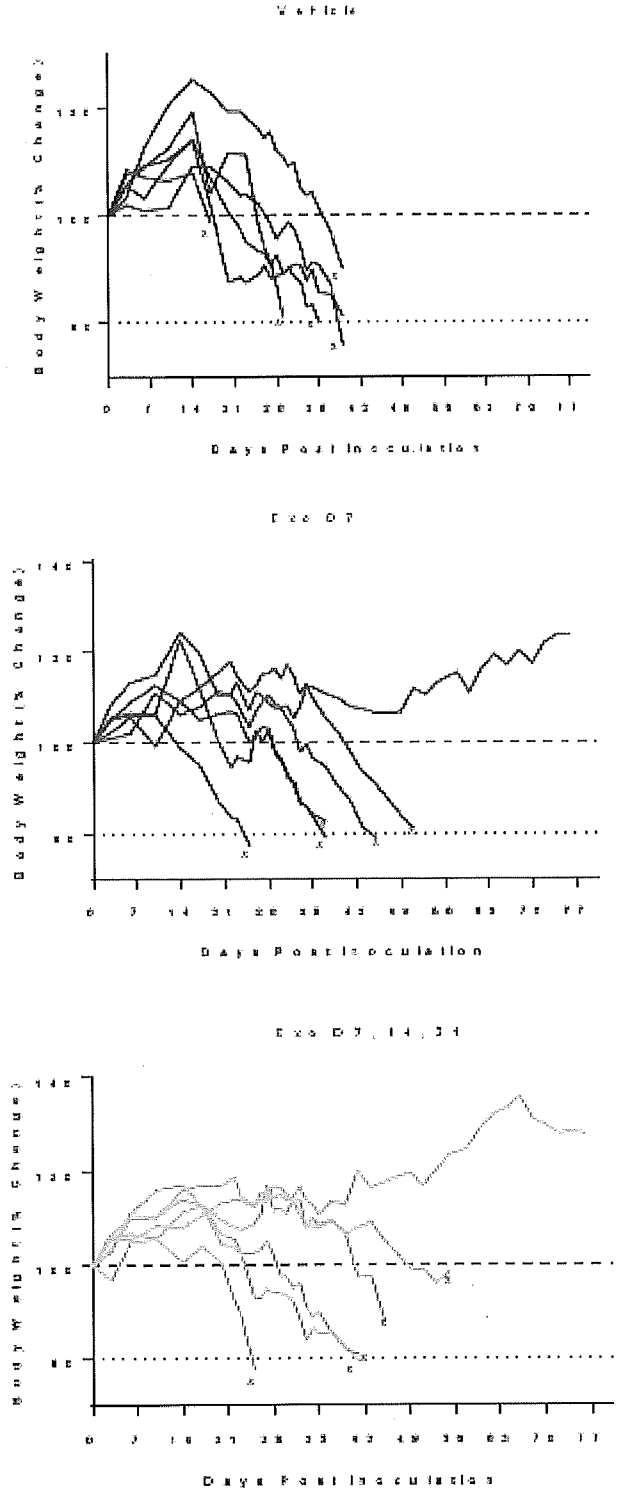


FIG. 45

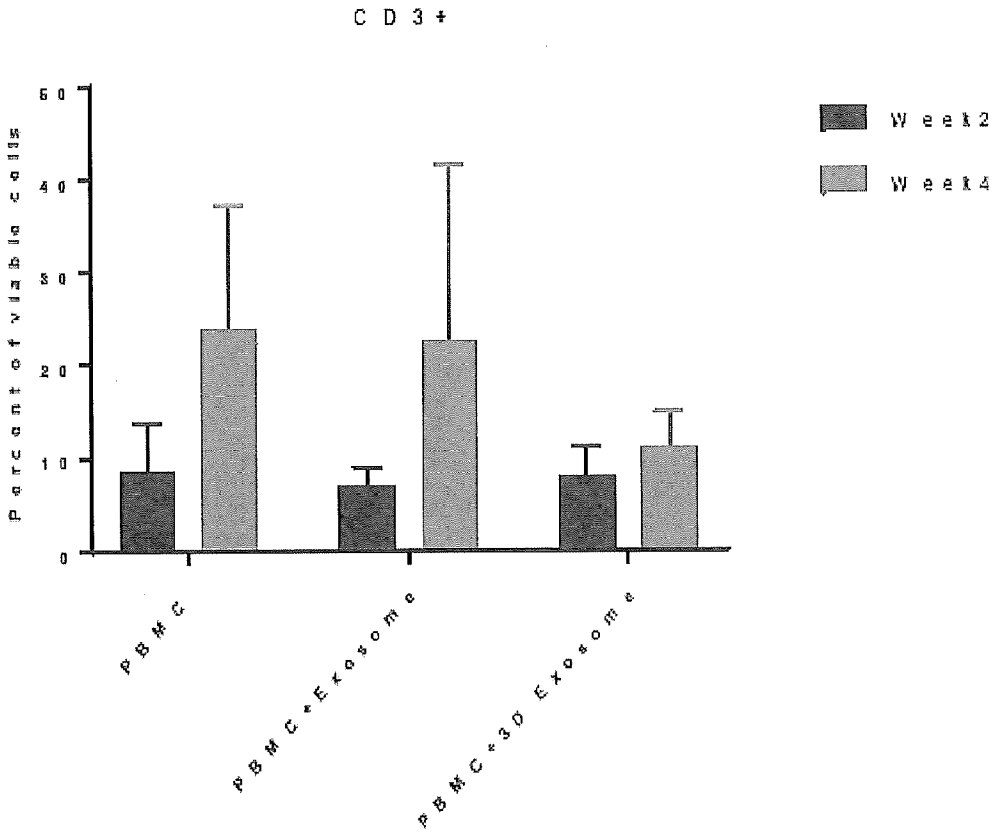

CD3+
Percent of viable cells
60
40
30
20
10
0
Week2
Week4
PBMC
PBMC+Exosome
PBMC+3D Exosome

FIG. 46

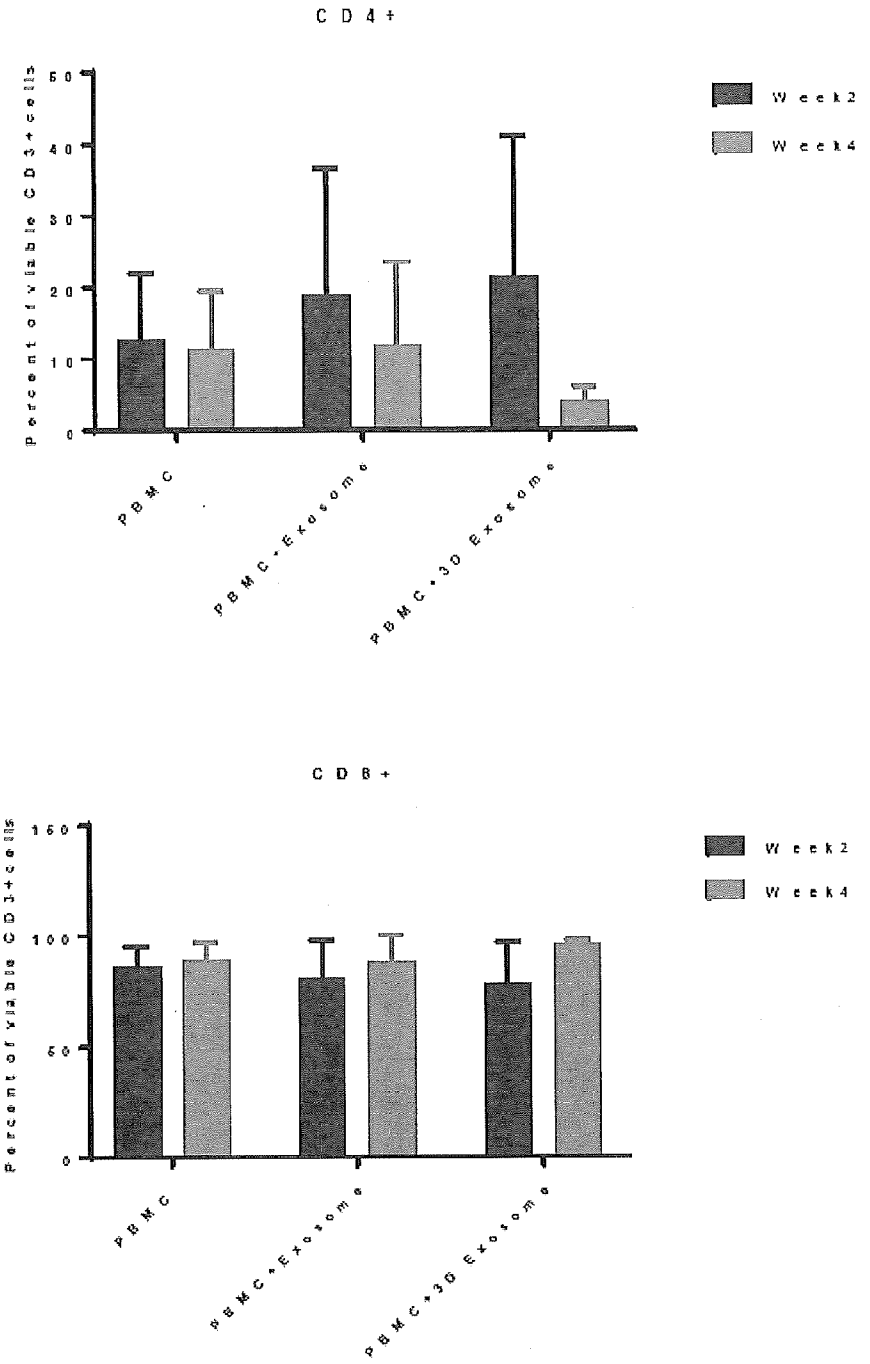

CD4+
Percent of viable CD3+cells
60
40
30
20
10
0
Week2
Week4
PBMC
PBMC+Exosome
PBMC+3D Exosome
CD8+
Percent of viable CD3+cells
150
100
50
0
Week2
Week4
PBMC
PBMC+Exosome
PBMC+3D Exosome

FIG. 47

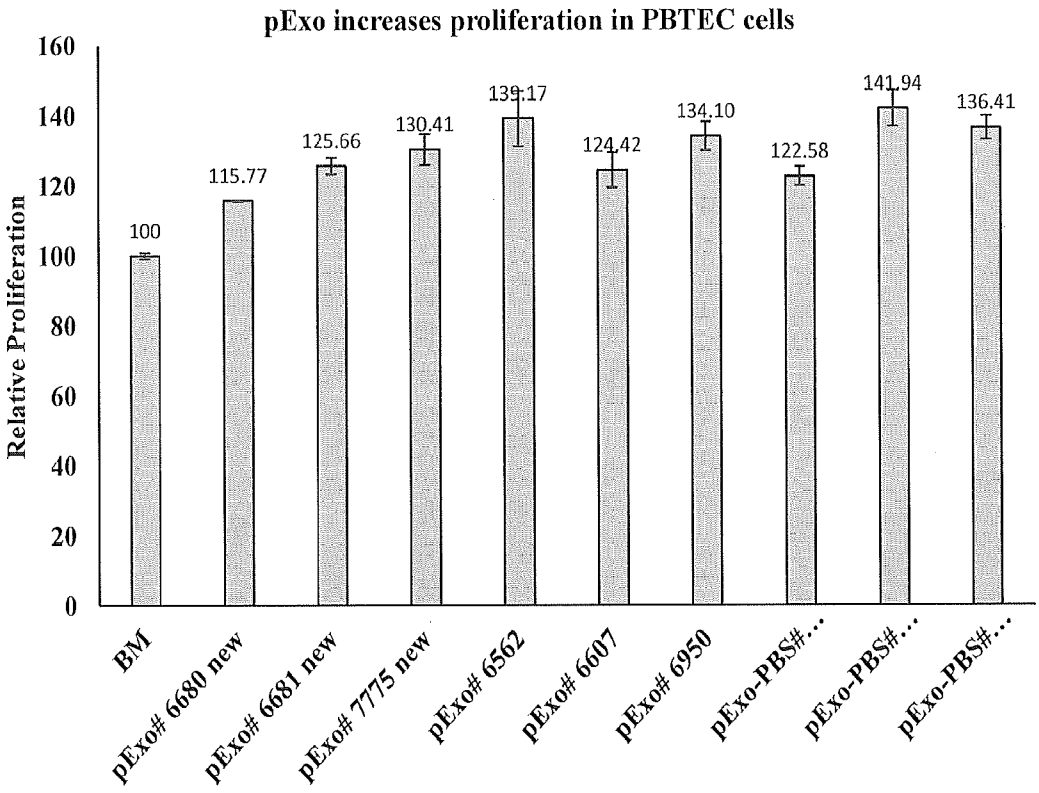

pExo increases proliferation in PBTEC cells
Relative Proliferation
160
140
120
100
80
60
40
20
0
100
115.77
125.66
130.41
139.17
124.42
134.10
122.58
141.94
136.41
BM
pExo# 6680 new
pExo# 6681 new
pExo# 7775 new
pExo# 6562
pExo# 6607
pExo# 6950
pExo-PBS#...
pExo-PBS#...
pExo-PBS#...

FIG. 48

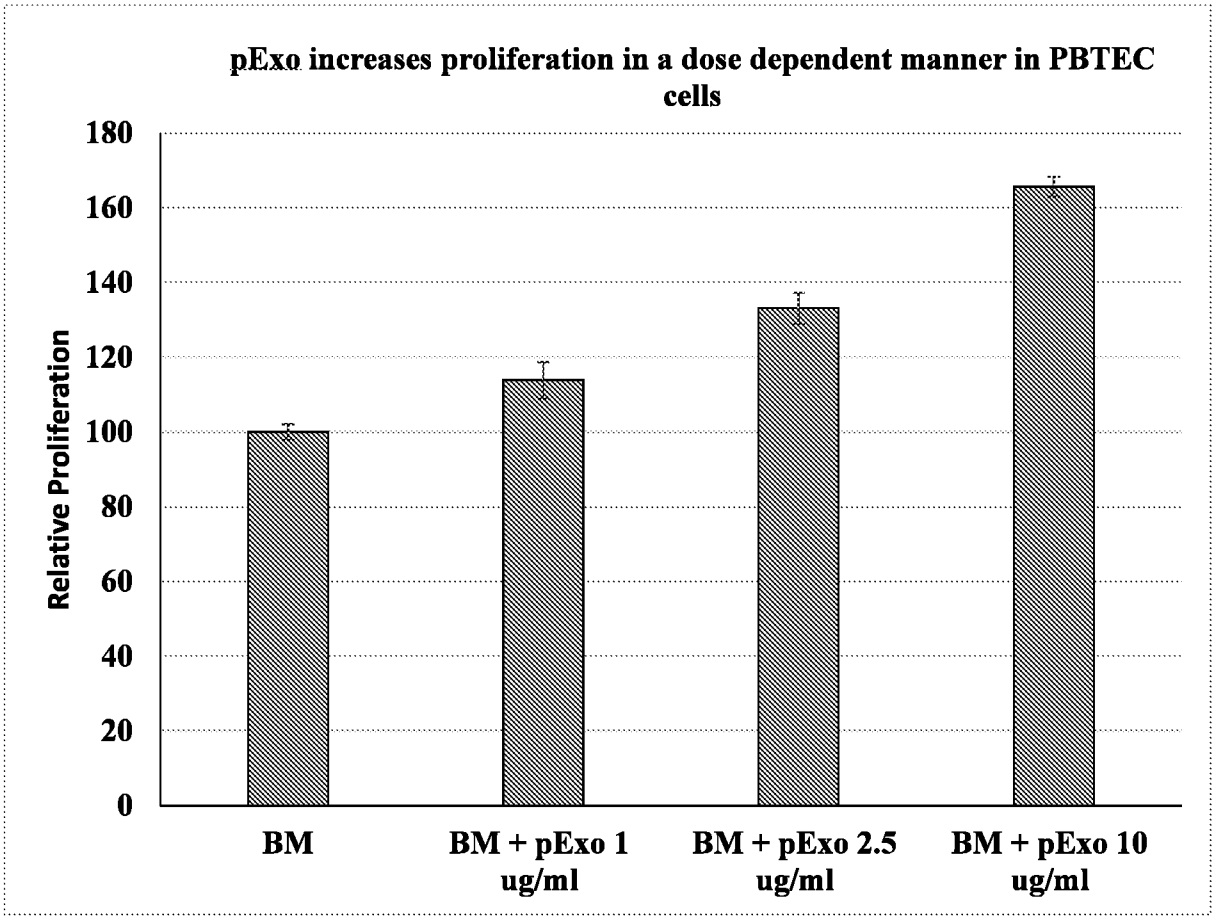

pExo increases proliferation in a dose dependent manner in PBTEC cells
Relative Proliferation
180
160
140
120
100
80
60
40
20
0
BM
BM + pExo 1 ug/ml
BM + pExo 2.5 ug/ml
BM + pExo 10 ug/ml

FIG. 49