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(54) Title: ADIPOSE DERIVED STEM CELL EXOSOMES AND USES THEREOF

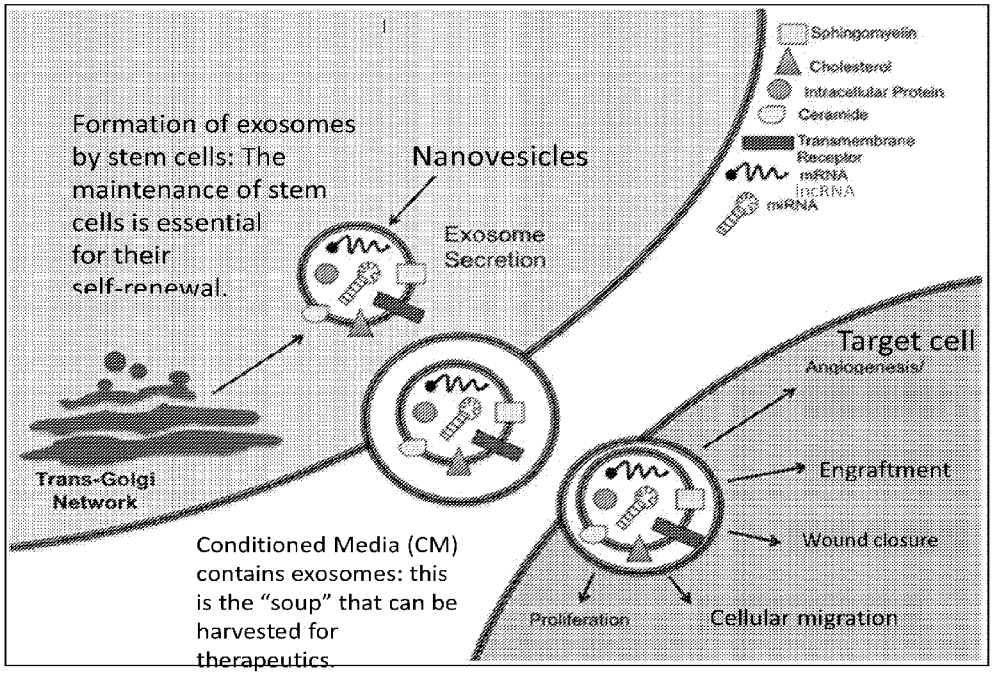


FIG. 1

(57) Abstract: Provided herein are exosomal compositions and exsosomal lncRNA compositions and formulations thereof. Also provided herein are methods of treating a wound in a subject in need thereof that can contain the step of administering an exosomal composition and/or exsosomal lncRNA compositions or formulation thereof to a wound in a subject in need thereof.

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ADIPOSE DERIVED STEM CELL EXOSOMES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of and priority to co-pending U.S. Provisional Patent Application No. 62/381,054, filed on August 30, 2016, entitled "ADIPOSE DERIVED STEM CELL EXOSOMES AND USES THEREOF," the contents of which is incorporated by reference herein in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under grant number HL 120954 awarded by the National Institutes of Health. The government has certain rights in the invention.

SEQUENCE LISTING

This application contains a sequence listing filed in electronic form as an ASCII.txt file entitled 292103-2780_ST25.txt, created on August 30, 2017. The content of the sequence listing is incorporated herein in its entirety.

BACKGROUND

There is a rapidly growing cohort of older people, including veterans with diabetes and a new cohort of wounded people, including veterans, with injuries that include non-healing ulcers from spinal cord injury, multiple traumatic injuries, and burns. In particular, veteran and similar populations are at risk for a staggering number of chronic wounds. As such, there exists an unmet need for improved compositions and treatments for chronic wounds.

SUMMARY

Described herein are pharmaceutical formulations that can include an amount of adipose stem cell derived exosomes, wherein the exosomes can have an amount of MALAT1 lncRNA; and a pharmaceutically acceptable carrier. The amount of adipose stem cell derived exosomes can be an effective amount sufficient to increase the rate of wound healing. In some aspects, the effective amount can range from about 0.001 pg to about 500 µg. In some aspects, the effective amount can range from about 9 µg/mL to about 20 µg/mL.

Also described herein are pharmaceutical formulations that can include an amount of MALAT1, VLDLR, or GAS5 lncRNA; and a pharmaceutically acceptable carrier. The amount

of MALAT1, VLDLR, or GAS5 lncRNA can be an effective amount sufficient to increase the rate of wound healing. In some aspects, the effective amount can range from about 0.001 pg to about 500 µg or more. In some aspects, the effective amount can range from about 0.001 pg/mL to about 500 µg/mL. In some aspects, the MALAT1 lncRNA can have a sequence that is about 95-100% identical to SEQ ID NO.: 1 or is a fragment of at least 20 contiguous nucleotides that are 95-100% identical to SEQ ID NO: 1, the VLDLR lncRNA can have a sequence that is about 90-100% identical to SEQ ID NO.: 2 or can be a fragment of at least 20 contiguous nucleotides that can be about 95-100% identical to SEQ ID NO: 2; and the GAS5 lncRNA can have a sequence that is 90-100% about identical to SEQ ID NO.: 3 or can be a fragment of at least 20 contiguous nucleotides that are 95-100% identical to SEQ ID NO: 3.

Also described herein, are methods of treating a wound in a subject in need thereof, the method that can include the step of administering an amount of a pharmaceutical formulation as previously described and described elsewhere herein. In some aspects, the amount of the pharmaceutical formulation can be administered topically. In some aspects, the amount of the pharmaceutical formulation can be administered directly to the wound. In some aspects, administration can occur on or more times. In some aspects, the wound can be an ischemic wound.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a diagram of stem cells secreting factors that can assist patient's wounded cells.

FIGS. 2A-2B show micrographic images demonstrating the results of a scratch assay to assess migration of young human dermal fibroblasts exposed to unconditioned medium.

FIG. 3 shows a graph demonstrating the cell count of the cells under conditioned (Figs. 4B, 4D, and 4F) or unconditioned medium (UCM) (Fig. 2B) at 18h.

FIGS. 4A-4F show micrographic images demonstrating the results of a scratch assay to assess migration of young human dermal fibroblasts exposed to increasing concentrations of human adipose derived stem cell conditioned medium (hADSC-CM).

FIG. 5 shows an *in vivo* assay using a rat ischemic wound model modified for Fisher 344 rats.

FIG. 6 shows a graph demonstrating the results from treatment of young ischemic wounds in a rat ischemic wound model as described and shown in reference to FIG. 5. Twenty-four Fisher rats underwent creation of a bipedicled ischemic flap with 6-mm excisional wounds, two ischemic and two nonischemic (control wound). Young adult (6month old) rats were divided into groups of 6, comparing human ADSC-CM obtained from

lean donors (Zenbio™). Twenty microliters of CM was applied topically daily to each wound beginning on the day of surgery. Digital photographs were taken every 4 days and wound area was measured using Image J. analysis revealed a statistically significant difference between control and lean conditioned media in ischemic wound sizes for days 8, 12, and 16 ($P < 0.001$). 100% of lean CM treated ischemic wounds were healed at day 20, 50% of control media.

FIG. 7 shows a graph demonstrating the results from treatment of young nonischemic wounds in a rat ischemic wound model as described and shown in reference to FIG. 5.

FIG. 8 shows a graph demonstrating the results of measuring the wound closure by electric cell-substrate impedance sensing (ECIS™).

FIG. 9 shows a graph demonstrating the results of measuring the wound closure by electric cell-substrate impedance sensing (ECIS™) where the cells were treated with 20 µg of exosomes and were tested alone or in the presence of 50 µM dynasore. Briefly, human dermal fibroblast (HDF) cells on the microelectrodes of the ECIS wells were killed with a high electrical voltage. Subsequently, the migration of viable cells onto the wounded microelectrodes was measured in real-time by electrical resistance in the presence of 20 µg/,L exosome, and then dynasore was added about 30 minutes later. The micrographs are normalized to the resistance before the wounding, N=3.

FIGS. 10A-10B show graphs demonstrating the RNA expression profile of several lncRNA and VLDLRs in conditioned medium (CM) and exosomes as detected by qRT-PCR.

FIGS. 11A-11B show micrographic images of the transfection control cells (Figs. 11A-11B).

FIG. 12 shows a graph demonstrating the quantitative results from the dermal fibroblast migration tests in Malat 1 and NEAT 1 knockdown in cells cultured in conditioned medium at 18 h as imaged in Figs. 11A-B and 13B, 13D, and 13F. Results are shown as % of control.

FIGS. 13A-13F show micrographic images of fibroblasts having knockdown of MALAT1 or NEAT 1 and the scramble control at 0 and 18 h and the effect of MALAT1 depletion on hADSC CM blocked cell migration. Briefly, young donor human dermal fibroblasts were plated in 6 well dishes and when 95% confluent, cells were scratched (3x) with a 200 µL pipette. Cells were treated with mitomycin C for about 2 hrs. Cell media from MALAT1 antisense oligonucleotide (ASO) treated or “scrambled” ASO treated or ADSC was added (about 2 mL) for about 18 h. After capturing images, migrating cells were counted in a field of the 3 scratch areas.

FIG. 14 shows a graph demonstrating the effect of conditioned media from adipose derived stem cells (ADSC) on cell migration in human dermal fibroblasts. Briefly, young

donor human dermal fibroblasts (HDF) were plated in 6 well dishes and when about 95% confluent, cells were scratched (3x) with a 200 µL pipette. Cells were treated with mitomycin C for about 2 h. Cell media from ZenBio ADSC was added (about 2 mL) for about 18 h. Unconditioned media (Lonza MSGMCD) was added as the control. After capturing images, migrating cells were counted in a field of the 3 scratch areas. PRISM™ analysis of the data indicated $P < 0.05$.

FIG. 15 shows a graph demonstrating the number of cells counted in a field of the 3 scratch areas of FIGS. 13A-13F. After capturing images, migrating cells were counted in a field of the 3 scratch areas. PRISM™ analysis of the data indicated $P < 0.01$.

FIG. 16 shows a graph demonstrating the effect of conditioned media from ADSC on HDF cell migration in an ECIS wounding assay. Briefly, HDF cells on the micro-electrods of the ECIS wells were killed with a high electrical voltage. Subsequently, the migration of viable cells onto the wounded microelectrodes was measured in real-time by electrical resistance in the presence of bFGF (about 400 nM), about 10 µg/mL exosomes, and about 20 µg/mL exosome respectively. The percentage of recovery was calculated by the difference in the resistance of each time point and the resistance of the first time point after wounding divided by the resistance before the wounding. The micrographs are representative of three ECIS wells at each time point.

FIG. 17 shows a graph demonstrating the effect of MALAT1 depletion on HDF cell migration as measured by an ECIS wound healing assay. Briefly, HDF cells on the micro-electrodes of the ECIS wells were killed with a high electrical voltage. Subsequently, the migration of viable cells into the wounded microelectrodes was measured in real-time by electrical resistance in the presence of bFGF (about 400 nM), 20 µg/mL exosome, about 20 µg/mL exosome-MALATI respectively. The micrographs are representative of three ECIS wells at each time point.

FIG. 18 shows a graph demonstrating the peak responses as cell migration rate from the HDF treatment groups described in relation to FIG. 17.

Further aspects of the present disclosure will be readily appreciated upon review of the detailed description of its various embodiments, described below, when taken in conjunction with the accompanying drawings.

DETAILED DESCRIPTION

Before the present disclosure is described in greater detail, it is to be understood that this disclosure is not limited to particular embodiments described, and as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described.

All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present disclosure is not entitled to antedate such publication by virtue of prior disclosure. Further, the dates of publication provided could be different from the actual publication dates that may need to be independently confirmed.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present disclosure. Any recited method can be carried out in the order of events recited or in any other order that is logically possible.

Embodiments of the present disclosure will employ, unless otherwise indicated, techniques of molecular biology, microbiology, nanotechnology, organic chemistry, biochemistry, botany and the like, which are within the skill of the art. Such techniques are explained fully in the literature.

Definitions

Unless otherwise specified the following definitions can apply herein to the following terms:

As used herein, "about," "approximately," and the like, when used in connection with a numerical variable, generally refers to the value of the variable and to all values of the

variable that are within the experimental error (e.g., within the 95% confidence interval for the mean) or within ± 10% of the indicated value, whichever is greater.

As used herein, “control” can refer to an alternative subject or sample used in an experiment for comparison purposes and included to minimize or distinguish the effect of variables other than an independent variable.

As used herein, “pharmaceutically acceptable carrier or excipient” can refer to a carrier or excipient that is useful in preparing a pharmaceutical formulation that is generally safe, non-toxic, and is neither biologically or otherwise undesirable, and includes a carrier or excipient that is acceptable for veterinary use as well as human pharmaceutical use. A “pharmaceutically acceptable carrier or excipient” as used in the specification and claims includes both one and more than one such carrier or excipient.

As used herein, “pharmaceutical formulation” can refer to the combination of an active agent, compound, or ingredient with a pharmaceutically acceptable carrier or excipient, making the composition suitable for diagnostic, therapeutic, or preventive use in vitro, in vivo, or ex vivo.

As used interchangeably herein, "subject," "individual," or "patient," refers to a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. The term “pet” includes a dog, cat, guinea pig, mouse, rat, rabbit, ferret, and the like. The term “farm animal” includes a horse, sheep, goat, chicken, pig, cow, donkey, llama, alpaca, turkey, and the like.

As used herein, “therapeutic” generally can refer to treating, healing, and/or ameliorating a disease, disorder, condition, or side effect, or to decreasing in the rate of advancement of a disease, disorder, condition, or side effect. The term also includes within its scope enhancing normal physiological function, palliative treatment, and partial remediation of a disease, disorder, condition, side effect, or symptom thereof.

As used herein, the terms "treating" and "treatment" can refer generally to obtaining a desired pharmacological and/or physiological effect. The effect may be prophylactic in terms of preventing or partially preventing a disease, symptom or condition thereof.

As used herein, “active agent” or “active ingredient” refers to a component or components of a composition to which the whole or part of the effect of the composition is attributed.

As used herein, “dose,” “unit dose,” or “dosage” refers to physically discrete units suitable for use in a subject, each unit containing a predetermined quantity of a collagen or fragment thereof, composition, or formulation described herein calculated to produce the desired response or responses in association with its administration.

As used herein, “administering” refers to an administration that is oral, topical, intravenous, subcutaneous, transcutaneous, transdermal, intramuscular, intra-joint, parenteral, intra-arteriole, intradermal, intraventricular, intracranial, intraperitoneal, intralesional, intranasal, rectal, vaginal, by inhalation, or via an implanted reservoir. The term “parenteral” includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional, and intracranial injections or infusion techniques.

As used herein, “effective amount” can refer to the amount of a composition described herein or pharmaceutical formulation described herein that will elicit a desired biological or medical response of a tissue, system, animal, plant, protozoan, bacteria, yeast or human that is being sought by the researcher, veterinarian, medical doctor or other clinician. The “effective amount” can be an amount of a composition or compound that can increase rate of wound healing, including ischemic wound healing, increase angiogenesis in a wound (including but not limited to ischemic wounds), and/or increase cell migration, proliferation, and/or differentiation of skin cells. The term “effective amount” can also include “effective concentrations,” which are concentrations of composition or compound described herein effective to increase rate of wound healing, including ischemic wound healing, increase angiogenesis in a wound (including but not limited to ischemic wounds), and/or increase cell migration, proliferation, and/or differentiation of skin cells.

As used herein, “anti-infective” refers to compounds or molecules that can either kill an infectious agent or inhibit it from spreading. Anti-infectives include, but are not limited to, antibiotics, antibacterials, antifungals, antivirals, and antiprotozoals.

As used herein, “aptamer” refers to single-stranded DNA or RNA molecules that can bind to pre-selected targets including proteins with high affinity and specificity. Their specificity and characteristics are not directly determined by their primary sequence, but instead by their tertiary structure.

As used herein, “concentrated” refers to a molecule, including but not limited to a polynucleotide, peptide, polypeptide, protein, antibody, or fragments thereof, that is distinguishable from its naturally occurring counterpart in that the concentration or number of molecules per volume is greater than that of its naturally occurring counterpart.

As used herein, “chemotherapeutic agent” or “chemotherapeutic” refer to a therapeutic agent utilized to prevent or treat cancer.

As used herein, “diluted” refers to a molecule, including but not limited to a polynucleotide, peptide, polypeptide, protein, antibody, or fragments thereof, that is distinguishable from its naturally occurring counterpart in that the concentration or number of molecules per volume is less than that of its naturally occurring counterpart.

As used herein, "identity," is a relationship between two or more polypeptide sequences, as determined by comparing the sequences. In the art, "identity" also refers to the degree of sequence relatedness between polypeptide as determined by the match between strings of such sequences. "Identity" can be readily calculated by known methods, including, but not limited to, those described in (Computational Molecular Biology, *Lesk, A. M.*, Ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, *Smith, D. W.*, Ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, *Griffin, A. M., and Griffin, H. G.*, Eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, *von Heinje, G.*, Academic Press, 1987; and Sequence Analysis Primer, *Gribskov, M. and Devereux, J.*, Eds., M Stockton Press, New York, 1991; and *Carillo, H., and Lipman, D.*, SIAM J. Applied Math. 1988, 48: 1073. Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity are codified in publicly available computer programs. The percent identity between two sequences can be determined by using analysis software (*e.g.*, Sequence Analysis Software Package of the Genetics Computer Group, Madison Wis.) that incorporates the *Needelman and Wunsch*, (J. Mol. Biol., 1970, 48: 443-453,) algorithm (*e.g.*, NBLAST, and XBLAST). The default parameters are used to determine the identity for the polypeptides of the present disclosure.

As used herein, "immunomodulator," refers to an agent, such as a therapeutic agent, which is capable of modulating or regulating one or more immune function or response.

As used herein, "isolated" means separated from constituents, cellular and otherwise, in which the polynucleotide, peptide, polypeptide, protein, antibody, or fragments thereof, are normally associated with in nature. A non-naturally occurring polynucleotide, peptide, polypeptide, protein, antibody, or fragments thereof, do not require "isolation" to distinguish it from its naturally occurring counterpart.

The term "molecular weight", as used herein, generally refers to the mass or average mass of a material. If a polymer or oligomer, the molecular weight can refer to the relative average chain length or relative chain mass of the bulk polymer. In practice, the molecular weight of polymers and oligomers can be estimated or characterized in various ways including gel permeation chromatography (GPC) or capillary viscometry. GPC molecular weights are reported as the weight-average molecular weight (M_w) as opposed to the number-average molecular weight (M_n). Capillary viscometry provides estimates of molecular weight as the inherent viscosity determined from a dilute polymer solution using a particular set of concentration, temperature, and solvent conditions.

As used herein, "negative control" refers to a "control" that is designed to produce no effect or result, provided that all reagents are functioning properly and that the experiment is

properly conducted. Other terms that are interchangeable with "negative control" include "sham," "placebo," and "mock."

As used herein, "patient" refers to an organism, host, or subject in need of treatment.

As used herein, "positive control" refers to a "control" that is designed to produce the desired result, provided that all reagents are functioning properly and that the experiment is properly conducted.

As used herein, "expression" refers to the process by which polynucleotides are transcribed into RNA transcripts. In the context of mRNA and other translated RNA species, "expression" also refers to the process or processes by which the transcribed RNA is subsequently translated into peptides, polypeptides, or proteins.

As used herein, "gene" refers to a hereditary unit corresponding to a sequence of DNA that occupies a specific location on a chromosome and that contains the genetic instruction for a characteristic(s) or trait(s) in an organism. "Gene" also refers to the specific sequence of DNA that is transcribed into an RNA transcript that can be translated into a polypeptide or be a catalytic or non-translated RNA molecule including but not limited to tRNA, siRNA, piRNA, miRNA, lncRNA, and shRNA.

As used herein, "long non-coding RNA" refers to non-coding RNA molecules containing about 200 or more nucleotides that are not translated to a protein.

As used herein, "paralog" can refer to a homologue produced via gene duplication of a gene. In other words, paralogs are homologues that result from divergent evolution from a common ancestral gene. A "functional paralog" can refer to a paralog that has a similar function or action.

As used herein, "orthologues" can refer to homologues produced by speciation followed by divergence of sequence but not activity in separate species. When speciation follows duplication and one homologue sorts with one species and the other copy sorts with the other species, subsequent divergence of the duplicated sequence is associated with one or the other species. Such species specific homologues are referred to herein as orthologues. A "functional orthologue" can refer to a orthologue that has a similar function or action.

As used herein, "homologue" can refer to a polypeptide (or polynucleotide) sequence that shares a threshold level (set forth elsewhere herein) of similarity and/or identity as determined by alignment of matching amino acids (or nucleic acids). Two or more polypeptides determined to be homologues are said to be homologues. Homology is a qualitative term that describes the relationship between polypeptide sequences that is based upon the quantitative similarity. A "functional homologue" can refer to a homologue that has a similar function or action.

As used herein, "conditioned media" can refer to cell culture media containing biologically active components released by, excreted by, secreted by, or otherwise obtained from cells and/or tissues that were previously cultured in the cell culture media.

Discussion

Chronically ill patents are faced with challenges, particularly when they develop recalcitrant wounds. A number of local and systemic factors impact wound healing include, but not limited to, oxygenation, infection, age, sex hormones, stress, diabetes, obesity, medications (e.g. chemotherapy), alcoholism, smoking and nutrition. The wound healing process incudes four integrated and overlapping phases: (1) homeostasis, (2) inflammation, (3) proliferation, and (4) tissue remodeling. These phases must occur rapidly and appropriately in the proper sequence and continue for a specific duration for wound healing to occur successfully. Investigations and clinical studies have provided a wealth of information about normal and impaired wound healing. In sum, oxygen levels are important for optimum wound healing. Hypoxia (low oxygen) stimulates the release of growth factors and angiogenesis, while oxygen is needed to sustain the process. Inflammation, a normal part of wound healing is important in the removal of microorganisms. In complete removal of bacteria and endotoxins can lead to infections at the injured skin surface, lengthening the inflammatory phase. Prolongation and escalation of the inflammatory phase contributes to a failure to heal. It also leads to increased levels of matrix metalloproteinases that can degrade the extracellular matrix. Systemic factors also contribute to impaired wound healing.

The needs of a healing wound are complex. Single and multiple factors play a role in the individual features to contribute to the overall outcome. Mesenchymal stem cells (MSC) derived from bone marrow can improve wound fibroblast migration *in vitro*. Co-cultured MSCs with fibroblasts in Boyden chambers that fibroblasts migrated toward MSCs in the chamber. To obtain bone marrow stem cells, invasive and painful techniques are required. As such, there still exists a need for non-bone marrow based wound healing techniques and compositions.

With the foregoing in mind, described herein are compositions and formulations containing exosomes secreted by adipose derived stem cells (ADSC) and/or one or more biologic factors contained within an adipose stem cell derived exosome. In some embodiments, the biologic factor can be a long coding RNA, such as MALAT1, linc-VLDLR, and/or GAS5. Also provided herein are methods of treating a wound that can include the step of administering an amount of a composition or formulation containing exosomes secreted by adipose derived stem cells and/or one or more biologic factors contained within an adipose stem cell derived exosome. The exosomes and other formulations provided herein can provide faster and/or more efficient wound healing than current techniques. Other compositions, compounds, methods, features, and advantages of the present disclosure will

be or become apparent to one having ordinary skill in the art upon examination of the following drawings, detailed description, and examples. It is intended that all such additional compositions, compounds, methods, features, and advantages be included within this description, and be within the scope of the present disclosure.

Exosomes and Exosomal lncRNAs

As shown in FIG. 1, adipose derived stem cells can produce and secrete exosomes. Provided herein are condition media containing adipose stem cell derived exosomes, adipose stem cell derived exosomes, and formulations thereof that can be effective for and used in the treatment of wounds. In embodiments, the wounds are recalcitrant wounds, such as ischemic wounds. In some embodiments, conditioned media can contain exosomes produced by adipose derived stem cells. In some embodiments, the exosomes can be purified and/or concentrated from the conditioned media. The exosomes, whether or not purified from the conditioned media, can contain one or more lncRNAs, including, but not limited to, MALAT1, linc-VLDR, GAS5, and/or functional homologues, paralogs, and/or orthologues thereof. In some embodiments, the most abundant lncRNA in one or more exosomes secreted by the adipose stem cells can be MALAT1. Techniques of determining the expression of lncRNAs, including MALAT1, are generally known in the art. The exosomes can be formed by isolating and culturing adipose derived stem cells for a period of time and collecting the formed exosomes secreted by the adipose derived stem cells by obtaining the culture medium and optionally purifying the exosomes from the culture medium. In some embodiments, the adipose derived stem cells are adipose derived mesenchymal stem cells. The period of time for culturing the adipose derived stem cells and collecting the exosomes can range from about 24 to 48 hours In some embodiments, the culture medium can be replaced with serum-free mesenchymal stem cell basal medium. As a non-limiting example, the serum-free mesenchymal stem cell basal medium can be MSC-BM-CD from Lonza Cat. No. #00190620). Other suitable media will be appreciated by those of ordinary skill in the art.

In addition to the techniques provided herein, the adipose derived stem cells, including adipose mesenchymal stem cells, can be obtained using techniques generally known in the art. In some embodiments, the adipose tissue can be collected by a suitable method (aspiration and/or liposuction) and the stromal vascular fraction of the adipose tissue can be isolated. Various techniques for obtaining the stromal vascular fraction and collection the stems cells within will be instantly appreciated by one of ordinary skill in the art. *See* e.g. Mailey, B. et al., 2014. Methods. Mol. Biol. 1210: 161-181. In addition to the techniques provided herein, the adipose derived stem cells can be cultured using techniques generally known in the art.

As previously described, the exosomes can include an amount of MALAT1 lncRNA or other lncRNAs. The exosomes can contain one or more lncRNAs, including, but not limited to, MALAT 1, linc-VLDLR, GAS5 and/or functional homologues, orthologues, or paralogues thereof. In some embodiments, the MALAT 1 lncRNA is the most abundant lncRNA in the exosomes. In some embodiments, the lncRNA can have a sequence about 95% to 100% identical to any one of SEQ ID NOs: 1-3 or a fragment of at least 20 contiguous nucleotides thereof. In some embodiments, the MALAT1, VLDLR, and/or GAS5 lncRNA can be isolated from the exosomes of adipose derived stem cells or *de novo* chemically synthesized and thus separated from the other components it associates with in nature.

The average size of the exosomes can range from 80 nm, to about 120 nm in diameter. The average hydrodynamic size of the exosomes can range from about 80 nm, to about 120 nm. The diameter or size of the exosomes can be measured, for example, by filtration via a membrane with a relevant size cutoff, electron microscopy, or SDS-PAGE or other biological assay. Hydrodynamic radius can be measured, for example, by laser diffraction or dynamic light scattering. Exosomes can fuse together and become larger, also, they can divide. Conditions governing the exact size of exosomes are currently unknown. Exosomes referred to in this application are identified not only by approximate size, but their expression of CD9, 63 and 81 by western blot.

Formulations Containing ADSC Exosomes and/or MALAT1 lncRNA

Also described herein are formulations, including pharmaceutical formulations, which can contain an amount of the conditioned media, exosomes and/or an amount of purified MALAT1, VLDLR, or GAS5 lncRNA described elsewhere herein. In some embodiments, the pharmaceutical formulation is the conditioned media containing the exosomes. The amount of the conditioned media, exosomes and/or an amount of purified MALAT1, VLDLR, or GAS5 lncRNA can be an effective amount. The amount of the conditioned media, exosomes and/or an amount of purified MALAT1, VLDLR, or GAS5 lncRNA can be effective to stimulate would healing and/or increase the rate of wound healing, including but not limited to increasing ischemic wound healing or rate thereof.

Formulations, including pharmaceutical formulations can be formulated for delivery via a variety of routes, including but not limited to topical, subcutaneously, and/or intravenously, and can contain a pharmaceutically acceptable carrier. Techniques and formulations generally can be found in *Remmington's Pharmaceutical Sciences*, Meade Publishing Co., Easton, Pa. (20^{th} Ed., 2000), the entire disclosure of which is herein incorporated by reference. For systemic administration, an injection is useful, including intramuscular, intravenous, intraperitoneal, and subcutaneous, transdermal, and topical. For

injection, the therapeutic compositions of the invention can be formulated in liquid solutions, for example in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the exosomes can be formulated in solid form (e.g. lyophilized) and redissolved or suspended immediately prior to use. Formulations, including pharmaceutical formulations, of the exosomes can be characterized as being at least sterile and pyrogen-free. These formulations include formulations for human and veterinary use.

The pharmaceutical formulations can include a pharmaceutically acceptable carrier. Suitable pharmaceutically acceptable carriers include, but are not limited to water, salt solutions, alcohols, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatin, carbohydrates such as lactose, amylose or starch, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid esters, hydroxyl methylcellulose, and polyvinyl pyrrolidone, which do not deleteriously react with exosomes and/or an amount of purified MALAT1, VLDLR, or GAS5 lncRNA. In some embodiments, the pharmaceutically acceptable carrier can be the cell culture medium that the adipose derived stem cells are cultured in (the conditioned media). Thus, in these embodiments, the conditioned media is the pharmaceutical formulation.

The pharmaceutical formulations can be sterilized, and if desired, mixed with auxiliary agents, such as platelet rich plasma, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, and the like which do not deleteriously react with the exosomes and/or an amount of purified MALAT1, VLDLR or GAS5 lncRNA.

A pharmaceutical formulation can be formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic, and stored at -80°C.

Formulations, including pharmaceutical formulations, suitable for injectable use can include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For

intravenous administration, suitable carriers can include physiological saline, bacteriostatic water, Cremophor EM™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). Injectable pharmaceutical formulations can be sterile and can be fluid to the extent that easy syringability exists. Injectable pharmaceutical formulations can be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, a pharmaceutically acceptable polyol like glycerol, propylene glycol, liquid polyethylene glycol, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In some embodiments, it can be useful to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, and sodium chloride in the composition.

Sterile injectable solutions can be prepared by incorporating any of the exosomes and/or an amount of purified lncRNAs described herein in an amount in an appropriate solvent with one or a combination of ingredients enumerated herein, as required, followed by filtered sterilization. Generally, dispersions can be prepared by incorporating exosomes and/or an amount of purified lncRNA into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated herein. In the case of sterile powders for the preparation of sterile injectable solutions, examples of useful preparation methods are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated can be used in the formulation, if needed. In some embodiments, an open wound is being treated and thus the penetrant is optional as application directly to the wound can provide sufficient contact with the wound to promote healing. Suitable penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fluidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the exosomes can be formulated into ointments, salves, gels, or creams as generally known in the art. In some embodiments, the exosomes and/or an amount of purified Malat1 lncRNA can be applied via transdermal delivery systems, which can slowly release the exosomes and/or an amount of purified Malat1 lncRNA for percutaneous absorption. Permeation enhancers can be used to facilitate transdermal penetration of the

active factors in the conditioned media, if present in the formulation, and/or exosomes. Transdermal patches are described in for example, U.S. Pat. No. 5,407,713; U.S. Pat. No. 5,352,456; U.S. Pat. No. 5,332,213; U.S. Pat. No. 5,336,168; U.S. Pat. No. 5,290,561; U.S. Pat. No. 5,254,346; U.S. Pat. No. 5,164,189; U.S. Pat. No. 5,163,899; U.S. Pat. No. 5,088,977; U.S. Pat. No. 5,087,240; U.S. Pat. No. 5,008,110; and U.S. Pat. No. 4,921,475

For parenteral administration (i.e., administration through a route other than the alimentary canal), the formulations described herein can be combined with a sterile aqueous solution that is isotonic with the blood of the subject. Such a formulation can be prepared by dissolving the active ingredient (e.g. the exosomes and/or an amount of purified lncRNA) in water containing physiologically-compatible substances, such as sodium chloride, glycine and the like, and having a buffered pH compatible with physiological conditions, so as to produce an aqueous solution, then rendering the solution sterile. The formulation can be presented in unit or multi-dose containers, such as sealed ampoules or vials. The formulation can be delivered by injection, infusion, or other means known in the art.

For transdermal administration, the formulations described herein can be combined with skin penetration enhancers, such as propylene glycol, polyethylene glycol, isopropanol, ethanol, oleic acid, N-methylpyrrolidone and the like, which increase the permeability of the skin to the nucleic acid vectors of the invention and permit the nucleic acid vectors to penetrate through the skin and into the bloodstream. The formulations and/or compositions described herein can be further combined with a polymeric substance, such as ethylcellulose, hydroxypropyl cellulose, ethylene/vinyl acetate, polyvinyl pyrrolidone, and the like, to provide the composition in gel form, which can be dissolved in a solvent, such as methylene chloride, evaporated to the desired viscosity and then applied to backing material to provide a patch.

Dosage forms

The exosomes, purified lncRNA, and formulations thereof described herein can be provided in unit dose form such as a tablet, capsule, single-dose injection, application or infusion vial, or as a predetermined dose in formulations described above. Where appropriate, the dosage forms described herein can be microencapsulated. The dosage form can also be prepared to prolong or sustain the release of any ingredient.

Effective Amounts

The formulations can contain an effective amount of the exosomes and/or an amount of purified lncRNA (effective for stimulating and/or increasing the rate of wound healing) described herein. In some embodiments, the effective amount of the exosomes and/or an amount of purified MALAT1, VLDLR, or GAS5 lncRNA ranges from about 0.001 pg to about 500 µg or more of the exosomes and/or an amount of purified MALAT1 lncRNA described herein. In some embodiments, the effective amount of the conditioned media, exosomes

and/or an amount of purified MALAT1, VLDLR, or GAS5 lncRNA can be about 20 μg/mL. In some embodiments, the effective amount of the exosomes and/or an amount of purified MALAT1 lncRNA described herein can range from about 0.001 pg, 0.01 pg, 0.1 pg, 0.001 μg, 0.01 μg, 0.1 μg, 1 μg, 2 μg, 3 μg, 4 μg, 5 μg, 6 μg, 7 μg, 8 μg, 9 μg, 10 μg, 15 μg, 20 μg, 25 μg, 30 μg, 35 μg, 40 μg, 45 μg, 50 μg, 55 μg, 60 μg, 65 μg, 70 μg, 75 μg, 80 μg, 85 μg, 90 μg, 95 μg, 100 μg, 125 μg, 150 μg, 175 μg, 200 μg, 225 μg, 250 μg, 275 μg, 300 μg, 350 μg, 400 μg, 450 μg to about 500 μg or more or from about 0.01 pg/mL, 0.1, pg/mL, 1 μg/mL, 2 μg/mL, 3 μg/mL, 4 μg/mL, 5 μg/mL, 6 μg/mL, 7 μg/mL, 8 μg/mL, 9 μg/mL, 10 μg/mL, 15 μg/mL, 20 μg/mL, 25 μg/mL, 30 μg/mL, 35 μg/mL, 40 μg/mL, 45 μg/mL, 50 μg/mL, 55 μg/mL, 60 μg/mL, 65 μg/mL, 70 μg/mL, 75 μg/mL, 80 μg/mL, 85 μg/mL, 90 μg/mL, 95 μg/mL, 100 μg/mL, 125 μg/mL, 150 μg/mL, 175 μg/mL, 200 μg/mL, 225 μg/mL, 250 μg/mL, 275 μg/mL, 300 μg/mL, 350 μg/mL, 400 μg/mL, 450 μg/mL to about 500 μg/mL. In some embodiments the effective amount can range from about 9 μg/mL to about 20 μg/mL. In some embodiments the effective amount can range from about 9 μg/mL. In some embodiments the effective amount can range from about 18 μg/mL. In some embodiments the effective amount can range from about 20 μg/mL.

In some embodiments, the effective amount of the exosomes and/or an amount of purified MALAT1 lncRNA described herein can range from about 0.001 pg, 0.01 pg, 0.1 pg, 0.001 μg, 0.01 μg, 0.1 μg, 1 μg, 2 μg, 3 μg, 4 μg, 5 μg, 6 μg, 7 μg, 8 μg, 9 μg, 10 μg, 15 μg, 20 μg, 25 μg, 30 μg, 35 μg, 40 μg, 45 μg, 50 μg, 55 μg, 60 μg, 65 μg, 70 μg, 75 μg, 80 μg, 85 μg, 90 μg, 95 μg, 100 μg, 125 μg, 150 μg, 175 μg, 200 μg, 225 μg, 250 μg, 275 μg, 300 μg, 350 μg, 400 μg, 450 μg to about 500 μg or more or from about 0.01 pg/mL, 0.1, pg/mL, 1 μg/mL, 2 μg/mL, 3 μg/mL, 4 μg/mL, 5 μg/mL, 6 μg/mL, 7 μg/mL, 8 μg/mL, 9 μg/mL, 10 μg/mL, 15 μg/mL, 20 μg/mL, 25 μg/mL, 30 μg/mL, 35 μg/mL, 40 μg/mL, 45 μg/mL, 50 μg/mL, 55 μg/mL, 60 μg/mL, 65 μg/mL, 70 μg/mL, 75 μg/mL, 80 μg/mL, 85 μg/mL, 90 μg/mL, 95 μg/mL, 100 μg/mL, 125 μg/mL, 150 μg/mL, 175 μg/mL, 200 μg/mL, 225 μg/mL, 250 μg/mL, 275 μg/mL, 300 μg/mL, 350 μg/mL, 400 μg/mL, 450 μg/mL to about 500 μg/mL per cm^2 of wound area. In some embodiments the effective amount can range from about 9 μg/mL to about 20 μg/mL per cm^2 of wound area. In some embodiments the effective amount can range from about 9 μg/mL per cm^2 of wound area. In some embodiments the effective amount can range from about 18 μg/mL per cm^2 of wound area. In some embodiments the effective amount can range from about 20 μg/mL per cm^2 of wound area. In some embodiments the effective amount can range from about 9 μg/mL to about 20 μg/mL per 3 cm^2 of wound area. In some embodiments the effective amount can range from about 9 μg/mL per 3 cm^2 of wound area. In some embodiments the effective amount can range from about 18 μg/mL per 3 cm^2 of wound area. In some embodiments the effective amount can range from about 20 μg/mL per 3 cm^2 of wound area.

In yet other embodiments, the effective amount of the conditioned media, exosomes and/or an amount of purified MALAT1, VLDLR, or GAS5 lncRNA can range from about 1% w/w to about 99% or more w/w, w/v, or v/v of the total formulation. In some embodiments, the effective amount of the conditioned media, exosomes and/or an amount of purified MALAT1, VLDLR, or GAS5 lncRNA can be effective at increasing the rate of wound healing, cell proliferation increasing angiogenesis in the wound, and tissue remodeling.

In some embodiments, the effective amount of the pharmaceutical formulation can range from about 0.001 pg, 0.01 pg, 0.1 pg, 0.001 µg, 0.01 µg, 0.1 µg, 1 µg, 2 µg, 3 µg, 4 µg, 5 µg, 6 µg, 7 µg, 8 µg, 9 µg, 10 µg, 15 µg, 20 µg, 25 µg, 30 µg, 35 µg, 40 µg, 45 µg, 50 µg, 55 µg, 60 µg, 65 µg, 70 µg, 75 µg, 80 µg, 85 µg, 90 µg, 95 µg, 100 µg, 125 µg, 150 µg, 175 µg, 200 µg, 225 µg, 250 µg, 275 µg, 300 µg, 350 µg, 400 µg, 450 µg to about 500 µg or more or from about 0.01 pg/mL, 0.1, pg/mL, 1 µg/mL, 2 µg/mL, 3 µg/mL, 4 µg/mL, 5 µg/mL, 6 µg/mL, 7 µg/mL, 8 µg/mL, 9 µg/mL, 10 µg/mL, 15 µg/mL, 20 µg/mL, 25 µg/mL, 30 µg/mL, 35 µg/mL, 40 µg/mL, 45 µg/mL, 50 µg/mL, 55 µg/mL, 60 µg/mL, 65 µg/mL, 70 µg/mL, 75 µg/mL, 80 µg/mL, 85 µg/mL, 90 µg/mL, 95 µg/mL, 100 µg/mL, 125 µg/mL, 150 µg/mL, 175 µg/mL, 200 µg/mL, 225 µg/mL, 250 µg/mL, 275 µg/mL, 300 µg/mL, 350 µg/mL, 400 µg/mL, 450 µg/mL to about 500 µg/mL per cm^2 of wound area In some embodiments, the effective amount of the pharmaceutical formulation can be about 20 µg/mL per about 3 cm^2 of wound area. In some embodiments the effective amount of the pharmaceutical formulation can range from about 0.01 mL to 5 mL or more per cm^2 of wound area.

In some embodiments the exosomes, exosomal lncRNAs, or other compound described herein can be used in a medicament for the treatment and/or promotion of wound healing.

Methods of Using the Exosomes and Exosomal lncRNAs

An amount, including an effective amount, of the conditioned media, exosomes, and/or an amount of purified lncRNA and formulations thereof described herein can be administered to a subject in need thereof. In some embodiments the subject in need thereof can have a wound. The wound can be a recalcitrant, chronic, and/or ischemic wound.

Administration of the exosomes and/or purified MALAT1 lncRNA is not restricted to a single route, but can encompass administration by multiple routes. For instance, exemplary administrations by multiple routes include, among others, a combination of dermal, transdermal, topical, intradermal and intramuscular administration, or intradermal and subcutaneous administration. Multiple administrations can be sequential or concurrent. Other modes of application by multiple routes will be apparent to the skilled artisan.

The pharmaceutical formulations can be administered to a subject by any suitable method that allows the exosomes and/or an amount of purified MALAT1 lncRNA to exert its effect on the subject *in vivo*. For example, the formulations and other compositions

described herein can be administered to the subject by known procedures including, but not limited to, by dermal, transdermal, topical, intravenous, and subcutaneous injection. Delivery can be by injection, infusion, catheter delivery, or some other means, such as by salve, lotion, gel, or spray.

In embodiments, the exosomes and/or purified MALAT1 lncRNA, or formulation thereof can be effective to treat a wound in a subject. In some embodiments, administration of the exosomes, lncRNAs, or formulations thereof can increase the rate of wound healing in the subject. In some embodiments, administration of the exosomes, lncRNas, or formulations thereof can increase angiogenesis of the wound in the subject. It can increase cell proliferation, and tissue remodeling.

The exosomal and lncRNA compositions and formulations thereof provided herein can be administered to the subject one or more times. Where administration occurs more than once the time period between each does can each independently range from minutes, to hours (e.g. 1, 2, 4, 6, 8, 10, 12 or more hours), days (e.g. 1,2, 3, 4, 5, 6, and/or 7 days), weeks (e.g. 1-52 weeks, or years (e.g. 1 to 2, 3, 4, 5, or more years) apart. Administration can occur during any life stage of the subject. Administration can be simultaneously or in series with other compounds or formulations (e.g. as a combination therapy). In some embodiments, administration can take place 1, 2, 4,6, 8, 10, 12 or more hours), days (e.g. 1,2, 3, 4, 5, 6, and/or 7 days), weeks (e.g. 1-52 weeks, or years (.g. 1 to 2, 3, 4, 5, or more years) after wound formation in non-healing and recalcitrant wounds.

Combination Therapy

The pharmaceutical formulations or other compositions described herein can be administered to a subject either as a single agent, or in combination with one or more other agents. Additional agents include but are not limited to DNA, RNA, amino acids, peptides, polypeptides, antibodies, aptamers, ribozymes, guide sequences for ribozymes that inhibit translation or transcription of essential tumor proteins and genes, hormones, immunomodulators, antipyretics, anxiolytics, antipsychotics, analgesics, antispasmodics, anti-inflammatories, anti-histamines, anti-infectives, and chemotherapeutics.

EXAMPLES

Now having described the embodiments of the present disclosure, in general, the following Examples describe some additional embodiments of the present disclosure. While embodiments of the present disclosure are described in connection with the following examples and the corresponding text and figures, there is no intent to limit embodiments of the present disclosure to this description. On the contrary, the intent is to cover all

alternatives, modifications, and equivalents included within the spirit and scope of embodiments of the present disclosure.

Example 1:

Introduction:

Chronically ill patients heal recalcitrant ulcerative wounds more slowly. Further, the Veterans Administration has a rapidly growing cohort of older veterans with diabetes and a new cohort of wounded veterans with injuries that include non-healing ulcers from spinal cord injury, multiple traumatic injuries and burns. Veteran populations are at risk for a staggering number of chronic wounds. Human adipose-derived stem cells (hADSC) play an important role in tissue regeneration and exosomes secreted by stem cells contribute to their paracrine signaling. A number of local and systemic factors impact wound healing including oxygenation, infection, age, and sex hormones, stress, diabetes, obesity, medications (e.g. chemotherapy), alcoholism, smoking, and nutrition. (8) The wound healing process includes four integrated and overlapping phases: hemostasis, inflammation, proliferation, and tissue remodeling (9). These must occur rapidly and appropriately in the proper sequence and continue for a specific duration for wound healing to occur successfully (10). Investigations and clinical studies have provided information about normal and impaired wound healing. In sum, oxygen levels are important for optimum wound healing. Hypoxia (low oxygen) stimulates the release of growth factors and angiogenesis, while oxygen is needed to sustain the process (11). Inflammation, a normal part of wound healing is important in the removal of microorganisms. Incomplete removal of bacteria and endotoxins can lead to infections at the injured skin surface, lengthening the inflammatory phase. Prolongation and escalation of the imnflammatory phase contributes to a failure to heal. It also leads to increased levels of matrix metalloproteinases that can degrade the extracellular matrix. Systemic factors also contribute to impaired wound healing (8).

The needs of a healing wound are complex and mediated by single and multiple factors that play roles in the individual features of healing that contribute to the overall outcome. Meshenchymal stem cells (MSC) derived from bone marrow can improve wound fibroblast migration *in vitro* (12). However, obtaining bone marrow is quite invasive and thus alternatives are desirable.

This Example describes compositions that can be effective as a therapy that can promote tissue regeneration and/or reverse the consequences of recalcitrant wounds. Additionally, this Example evaluates the role of lncRNA MALAT1 (metastasis-associated lung adenocarcinoma transcript 1), a lncRNA in exosomes that can be present in conditioned media from ADSC and ischemic wound healing. In some instances, conditioned medium (CM) from ADSC from normal (non-diseased) donors was used to evaluate the

effect of stem cell secreted factors on fibroblast migration. Also, animal models were used to assess the effect of ADSC derived exosomes and lncRNA MALAT1 on healing in a model of traumatic brain injury (13). Exosomes, specific nanovesicles that can be secreted by stem cells, were isolated and the ability of secreted stem cell factors present in the exosomes to modify (e.g. increase) cell migration was evaluated. Exosomes can include many cargo proteins, lipids, RNAs, and other molecules. Exosomes can evade immune rejection by the host and can modify cellular responses (14).

A secreted nanovesicle from adipose derived stem cells (human ADSC) was used in the therapy. Human ADSC release trophic factors that can stimulate the endogenous repair mechanisms in wounds, have immunomodulatory effects, and can respond to the microenvironment (1, 2, 3). These factors have been shown to heal wounds. However, hADSC research is flawed by the lack of standardization and delivery methods (4).

This Example can demonstrate the effectiveness of stem cell regenerative secreted factors, such as exosomes and various lncRNAs, to heal wounds. The secreted factors can be contained in exosomes, which can act in a paracrine manner (FIG. 1). In particular, this Example focuses on the role of a new class of RNA, long-noncoding RNA MALAT1, and its potential role in cell migration, proliferation and angiogenesis via its interaction with microRNAs and proteins (5). Two models are used to demonstrate the effects of exosomes on wound healing. Without being bound to theory or example, it is believed that the collection and topical application of exosomes from hADSC can be a better treatment for difficult skin wounds because they can be stored at low temperatures and made available to wound care in non-clinical settings.

Materials and Methods

Unless otherwise described the following procedures were at least used.

Adult Human Dermal Fibroblast (HDF) culture: Adult-HDF were purchased from ScienCell (Catalog #2320). HDF, isolated from human skin, were negative for HIV-1, HCV, mycoplasma, bacteria, yeast, and fungi. Cells were expanded in Fibroblast Medium (FM, Cat #2301) according to manufacturer's protocol. Cells were subcultured in poly-L-lysine-coated culture vessels as recommended. Medium was changed every three days after plating until cells were 70% confluent and every other day until cell were 90% confluent.

Adipose derived stem cell (ADSC) culture and conditioned media (CM): Human ADSC were purchased from ZenBio, Inc. Cells were negative for HIV-1, HIV-2, HTLC-1, HTLV-2, Hep-B, Hep-C, and mycoplasma. Human ADSC were isolated from subcutaneous adipose tissue of normal, non-diabetic donors between the ages of 18-60 years undergoing elective surgery. They were cultured according to manufacturer. For CM collection, cells were grown to 90% confluence, media was replaced with chemically defined serum-free

Mesenchymal Stem Cell Basal Media (MSCBM)(Lonza™), and CM was collected after about 48 hours.

Exosome Isolation: Exosomes were isolated using a two-step protocol. First, ExoQuick™ solution (SBI) was added to culture media at a volume of 1 to 5. Following centrifugation at 1,500 g for 30 min, the pellet was further processed. ExoCapTM (JSR Life Sciences) composite reagent containing magnetic beads for CD9, CD63 and CD81 was used to purify exosomes. Exosomes were eluted from beads using manufacturer's elution buffer and used in wound healing assays after washing twice with 500 HL washing buffer (15). To deplete MALAT from exosomes, cells were transfected with antisense oligonucleotides (ASO) as described (10). Scrambled ASO as described (16). Scrambled ASO were transfected as a control into ADSC, and CM was collected as described above. Exosomes were quantitated using a NanoSight LM10 (Malvern Instruments), and showed an average size of about 90-100 nm at a concentration of 1.1 x 10^8 per ml from 10^6 cells CM (15, 16).

Scratch assays: HDF were plated in 24 well plates. The cells were grown to confluence and three days after confluence were mechanically disrupted with a sterile 200 µL pipette tip with the use of a grid of 3 x 3 mm squares scratched with a pipette tip as described (13). The pre-incubation with mitomycin C (10 µg/ml) for about 2 hrs blocks further proliferation so that only migration is followed.

ECIS assays: The ECIS or electric cell-substrate impedance sensing instrument (Applied Biophysics) recorded the impedance on electrically wounded hHDF to detect the migratory response. Briefly, 200 µl of cell suspension were seeded per well of 8W1E ECIS array (5 × 10^4 cells per well) coated with about 1% gelatin. After cells were confluent in the incubator, the ECIS chambers were mounted to the ECIS system. Once the baseline leveled off, a 240 mA current with 60 KHz was applied to the cell-covered electrode for 30 seconds to kill the cells on the electrode (250 µm diameter), which resulted in dropping the impedance to around 2000. Arrays were then washed with medium under the microscope to remove any dead cells on the electrodes. Thereafter, the wound healing process was determined in real-time by measuring the recovery of electrical impedance, an indicator of the surrounding viable fibroblast cells migrating into the wounded area.

Rat Model of Ischemic Wound Healing: Young (6 month old) male Fischer 344 rats (National Institute on Aging, Bethesda, MD) were utilized to create an ischemic wound model (4). Full thickness excisional wounds were created in the center of a 10.5 x 3.5-cm flap (ischemic wounds) with a 6 mm punch biopsy and wound healing followed for about 2, 5, 7, 10, 14, and 21 days. Control (non-ischemic) full thickness wounds were created on either side of the ischemic flap for comparison. At the time of wounding and upon harvest, six rats were anesthetized, ischemic and non-ischemic wounds were digitally photographed, and wound sizes were determined 7. CM from ZenBio™ ADSC (20HI) or control (unconditioned)

media (about 20 µL) was applied to each wound daily. On day 0 and on day 2, 5, 7, 10, and 21 wound sizes were determined.

Statistical Analysis: PRISM-6™ software was used for statistical analysis with appropriate tests for comparisons including one way ANOVA and unpaired t-test. Data are shown as mean ± SEM. An n=3 is shown unless stated otherwise.

Results

The results are demonstrated in FIGS. 2A-18. FIGS. 2A-2B and 4A-4F show micrographic images demonstrating the results of a scratch assay to assess migration of young human dermal fibroblasts exposed to increasing concentrations of human adipose derived stem cell conditioned medium (hADSC-CM) (FIGS. 4A-4F) or unconditioned medium (FIGS. 2A-2B). FIG. 3 shows a graph demonstrating the cell count of the cells under conditioned (FIGS. 4B, 4D, and 4F) or unconditioned medium (UCM) (FIG. 2B) at about 18h. Briefly, CM was collected after about 48 h of exposure to hADSCs and stored at -80°C. Primary human dermal fibroblasts (HDFs) were grown to about 80% confluence and transferred to low serum DMEM for about 24 h. Proliferation was inhibited with mitomycin C and monolayers of HDF were scratched with a yellow pipette tip. Cultures were treated with ADSC-CM at increasing concentrations as shown in FIGS. 3-4F. Cell migration images were captured at 0 hours and at about 18h.

FIG. 5 shows a cartoon demonstrating a rat ischemic wound model for Fisher 344 rats. The inset demonstrates placement of a silicone sheet. The 6 mm wounds are ischemic. Lateral wounds are not ischemic.

FIGS. 6-7 show graphs demonstrating the results from treatment of wounds in a rat ischemic wound model as described and shown in reference to FIG. 5. Briefly, Twenty-four Fisher rats underwent creation of a bipedicled ischemic flap with 6-mm excisional wounds, two ischemic and two nonischemic (control wound). Rats were divided into groups of 6, comparing human ADSC-CM obtained from lean donors (ZenBio). Twenty microliters of CM was applied topically daily to each wound beginning on the day of surgery. Digital photographs were taken about every 4 days and wound area measured using Image J. Analysis revealed a statistically significant difference between control and lean conditioned media in ischemic wound sizes for days 8, 12 and 16 ($p < 0.001$). 100% of lean CM treated ischemic wounds were healed at day 20, 50% of control media. (6)

FIGS. 8-9 show graphs demonstrating the results of measuring the wound closure by electric cell-substrate impedance sensing (ECIS™). Normalized resistance over a time course for young HDFs exposed to two concentrations of purified exosomes (µg/ml) vs the hFGF (400ng/ml) control, or control media. At zero time cells were “wounded” and additions were made. Resistance was followed for 30 hrs. The lower dose of the exosomes (about 9.25 µg/mL) increased resistance (cell migration) at the same rate as hFGF. The higher

dose (about 18.5 µg/mL) increased resistance in a dose responsive manner, indicating that exosomes alone, were capable of enhancing wound healing in this in vitro model (n=2). In FIG. 9, 20 µg of exosomes were tested alone or in the presence of 50 µM Dynasore. Dynasore blocked the cell migratory effect of exosomes (n=2).

FIGS. 10A-10B show graphs demonstrating the RNA expression profile of several lncRNA and VLDLRs in conditioned medium (CM) and exosomes as detected by qRT-PCR. Figs. 10A-10B show the relative quantification (RQ) of the RNA. Sc-ADSCn indicates normal subcutaneous stem cells. Ex indicates exosomes isolated from CM. Other lncRNA profiles of exosomes from ADSCs have also been examined.[7]

FIGS. 11A-13F demonstrate the results of antisense oligonucleotides (ASO) treatment on cells in CM as determined by a scratch assay. FIGS. 11A-11B and 13A-13D show micrographic images of the transfection control cells (FIGS. 11A-11B) and cells having knockdown of MALAT1 or NEAT 1 and the scramble control at 0 and 18h (FIGS. 13A-13F). ADSC were treated with 500 nm of ASO (obtained via MTA from ISIS Pharmaceuticals). After 48 hrs, cells were switched to a serum free media and CM was collected for another 48 hrs. FIG. 12 shows a graph demonstrating the quantitative results from the dermal fibroblast migration tests in MALAT1 and NEAT 1 knockdown in cells cultured in conditioned medium at 18 h as imaged in FIGS. 11A-B and 13B, 13D, and 13F. Results are shown as % of control.

FIG. 14 shows a graph demonstrating the effect of conditioned media from adipose derived stem cells (ADSC) on cell migration in human dermal fibroblasts. Briefly, young donor human dermal fibroblasts (HDF) were plated in 6 well dishes and when about 95% confluent, cells were scratched (3x) with a 200 µL pipette. Cells were treated with mitomycin C for about 2 h. Cell media from ZenBio ADSC was added (about 2 mL) for about 18 h. Unconditioned media (Lonza MSCMCD) was added as the control. After capturing images, migrating cells were counted in a field of the 3 scratch areas. PRISM™ analysis of the data indicated $P < 0.05$.

FIG. 15 shows a graph demonstrating the number of cells counted in a field of the 3 scratch areas of FIGS. 13A-13F. After capturing images, migrating cells were counted in a field of the 3 scratch areas. PRISM™ analysis of the data indicated $P < 0.01$.

FIG. 16 shows a graph demonstrating the effect of conditioned media from ADSC on HDF cell migration in an ECIS wounding assay. Briefly, HDF cells on the micro-electrods of the ECIS wells were killed with a high electrical voltage. Subsequently, the migration of viable cells onto the wounded microelectrodes was measured in real-time by electrical resistance in the presence of bFGF (about 400 nM), about 10 µg/mL exosomes, and about 20 µg/mL exosome respectively. The percentage of recovery was calculated by the difference in the resistance of each time point and the resistance of the first time point after

wounding divided by the resistance before the wounding. The micrographs are representative of three ECIS wells at each time point.

FIG. 17 shows a graph demonstrating the effect of MALAT1 depletion on HDF cell migration as measured by an ECIS wound healing assay. Briefly, HDF cells on the micro-electrodes of the ECIS wells were killed with a high electrical voltage. Subsequently, the migration of viable cells into the wounded microelectrodes was measured in real-time by electrical resistance in the presence of bFGF (about 400 nM), 20 µg/mL exosome, about 20 µg/mL exosome-MALATI respectively. The micrographs are representative of three ECIS wells at each time point.

FIG. 18 shows a graph demonstrating the peak responses as cell migration rate from the HDF treatment groups described in relation to FIG. 17.

Conditioned media from ADSC can promote HDF cell migration. Fibroblasts interact with their environment to produce a migratory mode in wound healing. There are complex networks that occur thorough signals that drive the processes in wound healing. The *in vitro* scratch assay is a method for analysis of cell migration when cells are manipulated with transfection or micro-injection to assess the effects of exogenous factors on migration of individual cells (12). In a probe for the secreted factors from ADSC that modulate cell migration, CM from ZenBio™ normal subcutaneous derived ADSC were collected. CM was collected under stringent conditions using a serum-free, clinical grade media. This 48 h collection was termed ZenBio™ CM. ZenBio™ CM was compared to control or unconditioned Lonza MSCBM media. CM was observed to increase the migration of HDF in the scratch assay by about 43% (FIG. 14). This result indicated that ZenBio™ CM contained factor(s) that increased cell migration. The scratch assays demonstrated that human dermal fibroblasts when challenged with a "scratch" migrated into the scratch faster with the conditioned media than with unconditioned media (FIGS. 2A-4F and 14). Hence, stem cells secrete factors that increase cell migration.

Conditioned Media from ADSC can heal ischemic wounds in vivo. The *in vitro* results using CM from ADSC suggested that fibroblast migration, which can play a role in wound closure, was accelerated. ZenBio™ hADSC CM was applied to rat ischemic wounds to determine if the *in vitro* results would translate *in vivo* using a rat *in vivo* model. ZenBio™ ADSC CM was applied to ischemic and non-ischemic wounds for about 20 days and the percent of wound closure that occurred in each wound was measured (FIGS. 6-7). The results demonstrated that in young adult rats, ZenBio™ hADSC CM accelerated closure of ischemic wounds. By day 4, about a 66% reduction of wound area in non-ischemic (control) wounds was observed. By day 8, this had increased to about an 86% wound area reduction, and by day 12, the non-ischemic wounds were healed. For ischemic wounds, ZenBio™ ADSC CM treated wounds were 10% closed on day 4, on day 8, about 30% wound closure

was measured, and by day 12, the wound area reduction was about 65% compared to only about 15% wound closure for the control (unconditioned) media treated wounds. Thus, ZenBio™ hADSC CM increased the ischemic wound closure by about 50% compared to non-ischemic wounds. This was actually more robust than expected based on the *in vitro* scratch assay results. The rat ischemic wound model reflected in vivo that conditioned media from hADSC, when applied at small quantities of 20 µl also resulted in healing ischemic wounds at a rate that mirrored the nonischemic or control wound (FIGS. 5-7).

Exosomes from CM simulated cell migration in ECIS assays. It was previously observed that ZenBio™ hADSC CM suppressed the recovery of rats injured with traumatic brain injury (17). In these experiments, CM was injected into the rats after injury and their ability to swim a water maze was improved. However, CM depleted of MALAT1 impaired this result. Here, the effect of depletion/reduction in CM was tested in young HDF. Depletion of MALAT1 in exosomes was accomplished using antisense oligonucleotides (ASO) to treat ZenBio™ hADSC with subsequent collection of CM. HDF trated with this CM resulted in reduction of cell migration of about 48% compared to CM from hADSC treated with the scrambled ASO (FIGS. 13A-13F and 15).

CM contains the ADSC secreted factors. The secretome of ADSC from CM contains a number of highly conserved proteins that have roles in angiogenesis, regeneration, and extracellular matrix remodeling (18). It was observed that exosomes isolated from CM contained in large concentrations of MALAT1 lncRNA (7, 20).

Exosomes can enhance cell migration. To evaluate the impact of CM exosomes on cell migration quantitatively, ECIS assays were employed (FIGS. 8-9 and 16). The concentration of exosomes applied was about equal to the number of exosomes found in the CM. For this assay, bFGF (about 400 nM) was used as a positive control as it is known to promote cell migration via the ERK1/2 abd Jnk pathways (21). The control (unconditioned media) and two doses of exosomes suspended in control media was then administered to injured to cells. FIG. 16 can demonstrate that the lower dose of the exosomes can increase recovery of transcellular electrical resistance (TER) similar to the effect of cells treated with bFGF. The higher concentration of exosomes was observed to enhance the recovery of TER faster and greater than the effect of bFGF. This indicated that exosomes promoted a strong effect on cell migration in the ECIS wound healing assay.

Dynasore can block the effect of exosomes. Extracellular exosomes have shown evidence that they can enter cells and deliver their cargo (19). The inhibitor, Dynasore, was added to determine if it blocked a clarthrin-and caveolin-dependent endocytosis of exosomes by the HDF. Dynasore (about 50 µM) addition to the exosome-containing media was observed to greatly attenuate cell migration (FIG. 9)

Exosomes isolated from MALAT1-depleted CM failed to enhance cell migration. The loss of function of MALAT1 in scratch assays was further demonstrated using exosomes derived from CM of hADSC treated with ASO. In this ECIS assay, resistance was measured in ohms. Treated cells were observed to have a significant increase of TER in comparison to cells treated with control media over a 16-hour course, which mimicked the effect of bFGF (about 400 nM) in the first 8 hours but displayed a greater rate of healing between hours 8 and 16. In cells treated with exosomes that were isolated from MALAT1 depleted hADSC, there was no observed significant effect on TER compared with control media (FIGS. 17-18). The result demonstrated importance of coexisting exosomes with MALAT1 in promoting cell migration and wound enclosure.

Discussion

The *in vivo* wound process is dynamic and consists of four continuous phases. In humans the relsease of pro-inflammatory cytokines such as bFGF is documented to occur prior to proliferation of fibroblasts within the wound bed to support angiogenesis (34). Treatement with CM from normal donor Zenbio™ ADSC was tested to determine if ischemic wounds heald at a greater rate than those treated with unconditioned or “control” media. It was observed that CM increased the rate of healing of HDF by a significant about 48%. This effect was mimicked in an animal model of ischemic wounds with results at days 12 and 16 that mirrored the effect of CM on cells. Levels of MALAT 1 in hADSC were also depleted using ASO. These hADSC produced CM that demonstrated about a 70 % to about 90 % reduction in MALAT1 content. This CM was tested in scratch assays and it was observed that CM with reduced MALAT1 content did not promote cell migration as well as the control media or CM from scrambled-ASO treated ADSC.

ECIS assays monitor real-time recovery of cell confluence after an electrically created wound by measureing the resistance of the wound area as resistance vs. time measured in these assays. ECIS assays demonstreated the ability of exosomes, isolated from CM, to increase migration of wounded cells. Migration was blocked using exosomes from CM of cells treated with MALAT1 ASO.

The diminished expression of MALAT1 by stem cells when treated with ASOs targeting this lncRNA resulted in a reduced secretion of MALAT1 into exosomes that was less than about 80% of the control. These depleted exosomes performed equally to “control” media in the ECIS assays. This followed the effect of MALAT1 ASO treated ADSC CM in the scratch assays. This data suggests that MALAT1 provided an important function in HDF migration in both assays evaluating CM and exosomes. Moreover, MALAT1 was taken up by the HDF as demonstrated by inhibition of migration when cells were treated with Dynasore, a blocker of clathrin- and caveolin-dependent endocytosis where dynamin-2 is the target (22, 23). The precise molecular mechanisms of MALAT1 action in determined. Its nuclear role is

in alternative splicing of pre-mRNA (20). The effect of exosomes released from human iPSC from mesenchymal stem cells was shown to promote collagen synthesis and angiogenesis *in vitro* and *in vivo* (24). Exosomes promoted proliferation and migration of human umbilical vein endothelial cells. This occurred in a dose dependent manner where about 100 µg/mL of exosomes were used. In this Example, ten-fold lower concentrations of exosomes were observed to promote migration.

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5

We claim:

1. A pharmaceutical formulation comprising an amount of adipose stem cell derived exosomes, wherein the exosomes comprise an amount of MALAT1 lncRNA; and

a pharmaceutically acceptable carrier.

2. The pharmaceutical formulation of claim 1, wherein the amount of adipose stem cell derived exosomes is an effective amount sufficient to increase the rate of wound healing.

3. The pharmaceutical formulation of claim 2, wherein the effective amount ranges from about 0.001 pg to about 500 µg.

4. The pharmaceutical formulation of any one of claims 2-3, wherein the effective amount ranges from about 9 µg/mL to about 20 µg/mL

5. A pharmaceutical formulation comprising an amount of MALAT1, VLDLR, or GAS5 lncRNA; and

a pharmaceutically acceptable carrier.

6. The pharmaceutical formulation of claim 5, wherein the amount of MALAT1, VLDLR, or GAS5 lncRNA is an effective amount sufficient to increase the rate of wound healing.

7. The pharmaceutical formulation of claim 6, wherein the effective amount can range from 0.001 pg to 500 µg or more.

8. The pharmaceutical formulation of claim 6, wherein the effective amount ranges from about 0.001 pg/mL to about 500 µg/mL.

9. The pharmaceutical formulation of any one of claims 5-8, wherein the MALAT1 lncRNA has a sequence that is about 95-100% identical to SEQ ID NO.: 1 or is a fragment of at least 20 contiguous nucleotides that are 95-100% identical to SEQ ID NO: 1, wherein the VLDLR lncRNA has a sequence that is 90-100% identical to SEQ ID NO.: 2 or is a fragment of at least 20 contiguous nucleotides that are 95-100% identical to SEQ ID NO: 2; and wherein the

GAS5 lncRNA has a sequence that is 90-100% identical to SEQ ID NO.: 3 or is a fragment of at least 20 contiguous nucleotides that are 95-100% identical to SEQ ID NO: 3.

10. A method of treating a wound in a subject in need thereof, the method comprising:

administering an amount of a pharmaceutical formulation as in any one of claims 1-9 to the subject in need thereof.

11. The method of claim 10, wherein the amount of the pharmaceutical formulation is administered topically.

12. The method of any one of claims 10-11, wherein the amount of the pharmaceutical formulation is administered directly to the wound.

13. The method of any one of claims 10-12, wherein the administration can occur on or more times.

14. The method of any one of claims 10-11, wherein the wound is an ischemic wound.

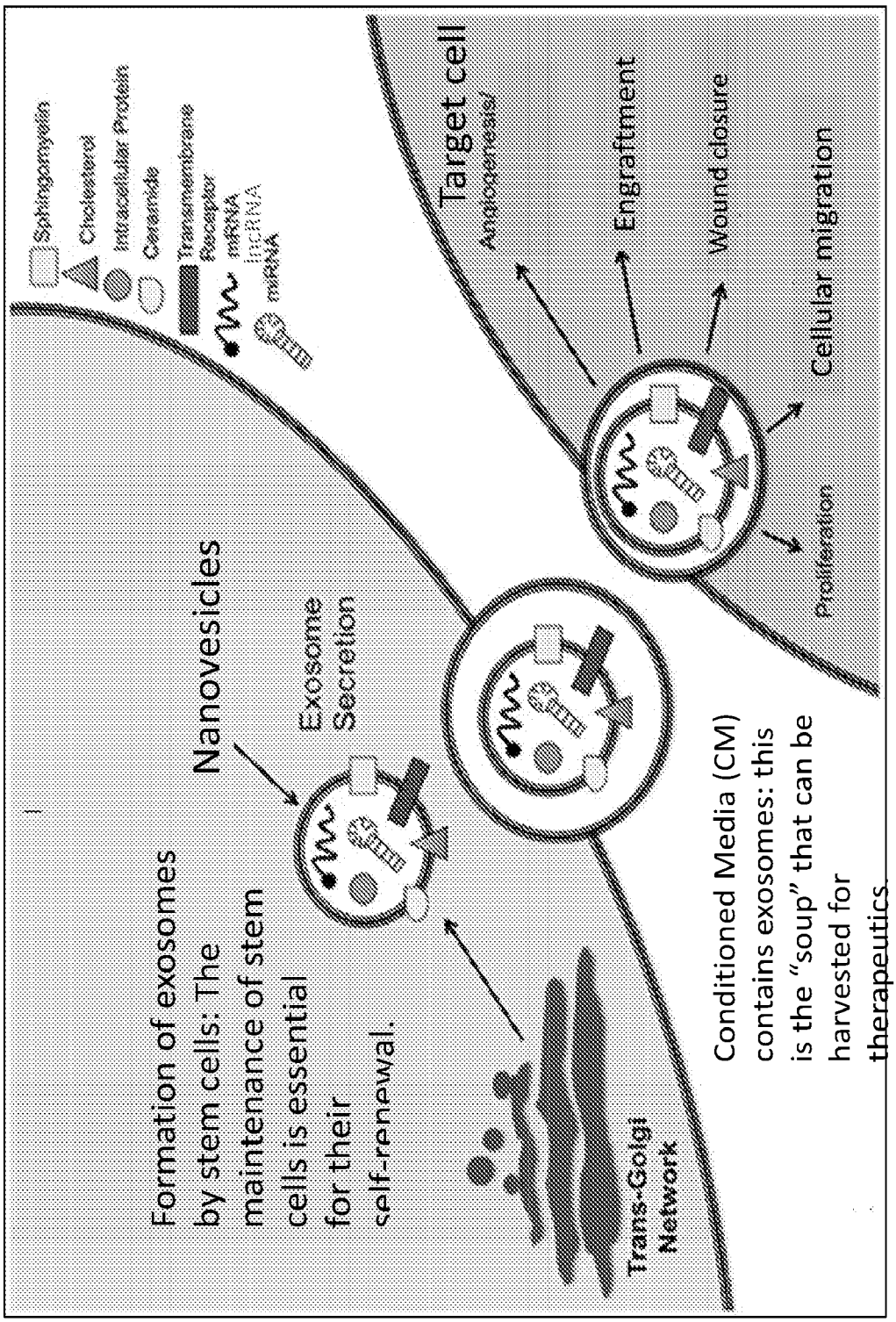
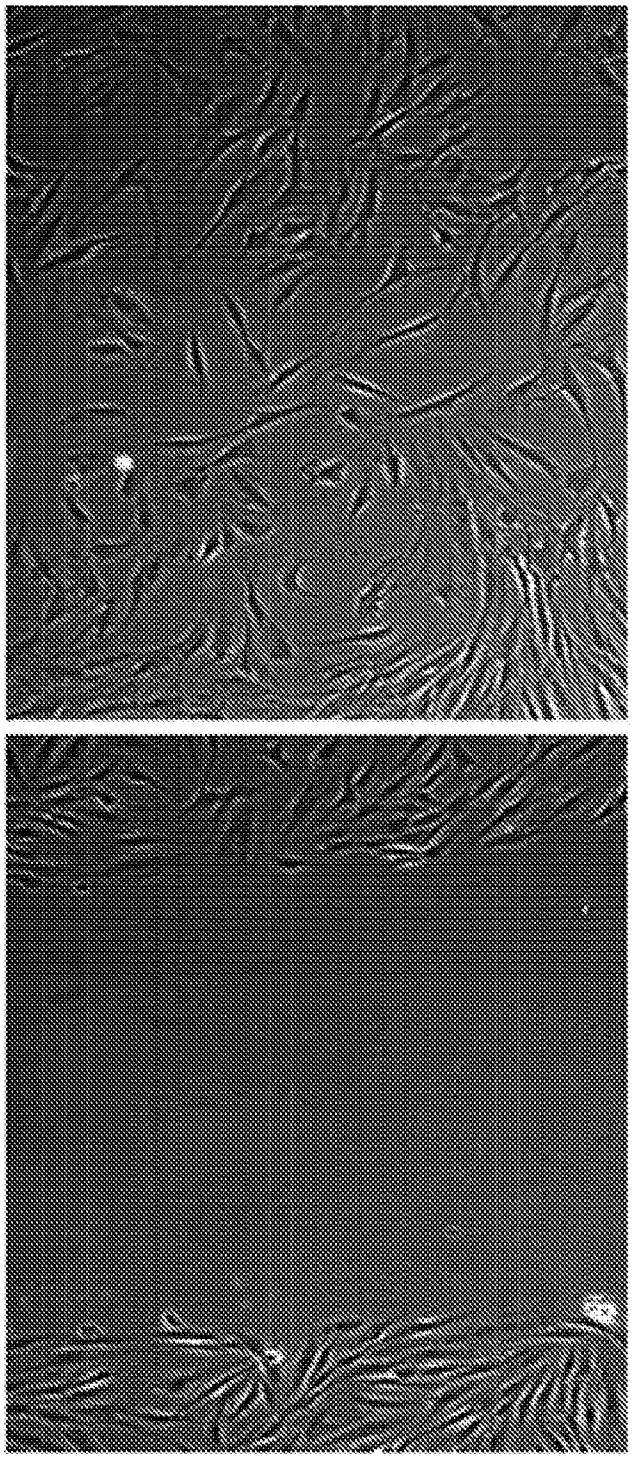

Sphingomyelin
Cholesterol
Intracellular Protein
Ceramide
Transmembrane Receptor
mRNA
lncRNA
miRNA
Target cell
Angiogenesis
Engraftment
Wound closure
Cellular migration
Proliferation
Nanovesicles
Exosome Secretion
Formation of exosomes by stem cells: The maintenance of stem cells is essential for their self-renewal.
Trans-Golgi Network
Conditioned Media (CM) contains exosomes: this is the "soup" that can be harvested for therapeutics

FIG. 1


FIG. 2B
18hr
FIG. 2A
0hr
Unconditioned Medium

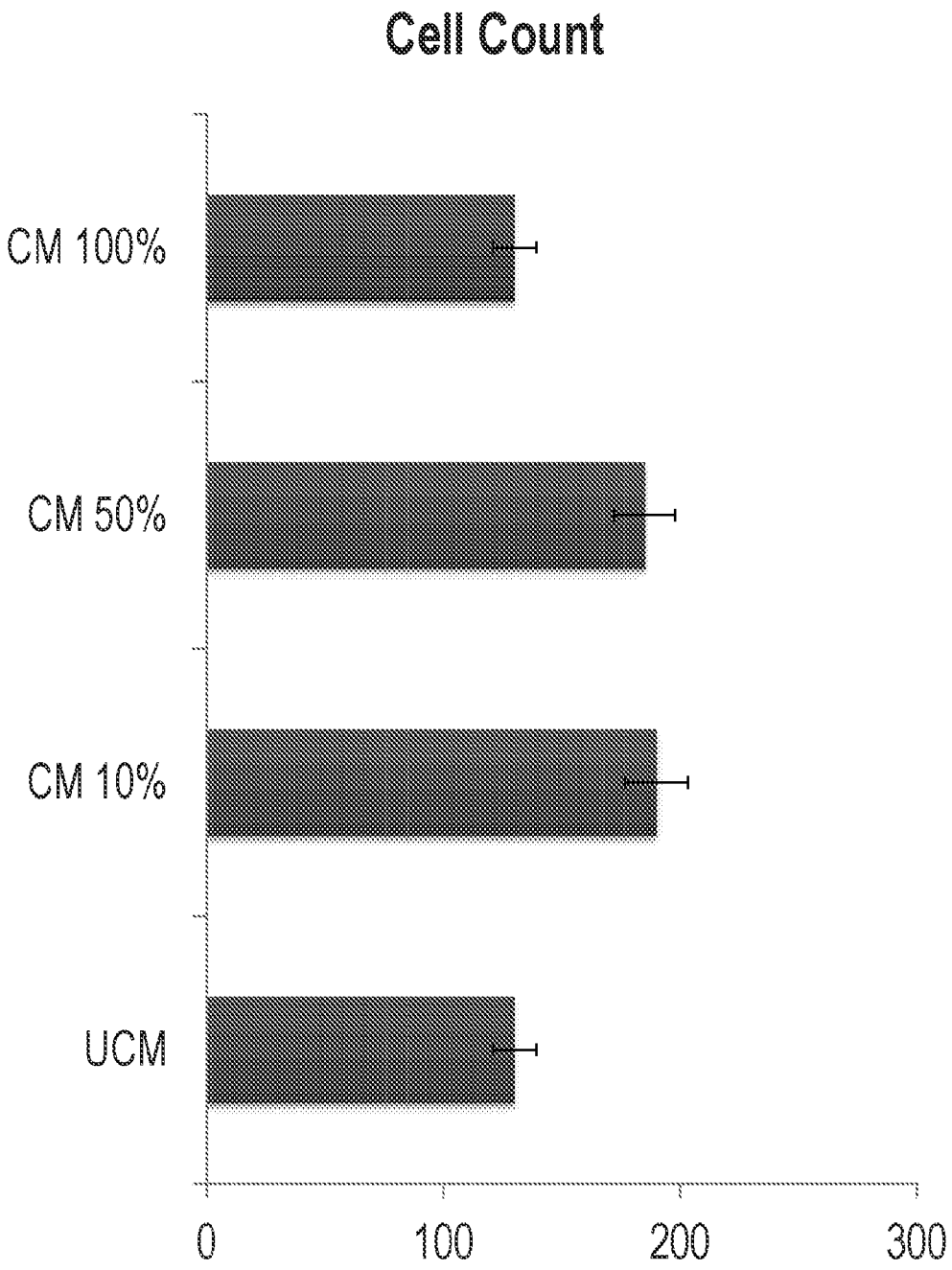
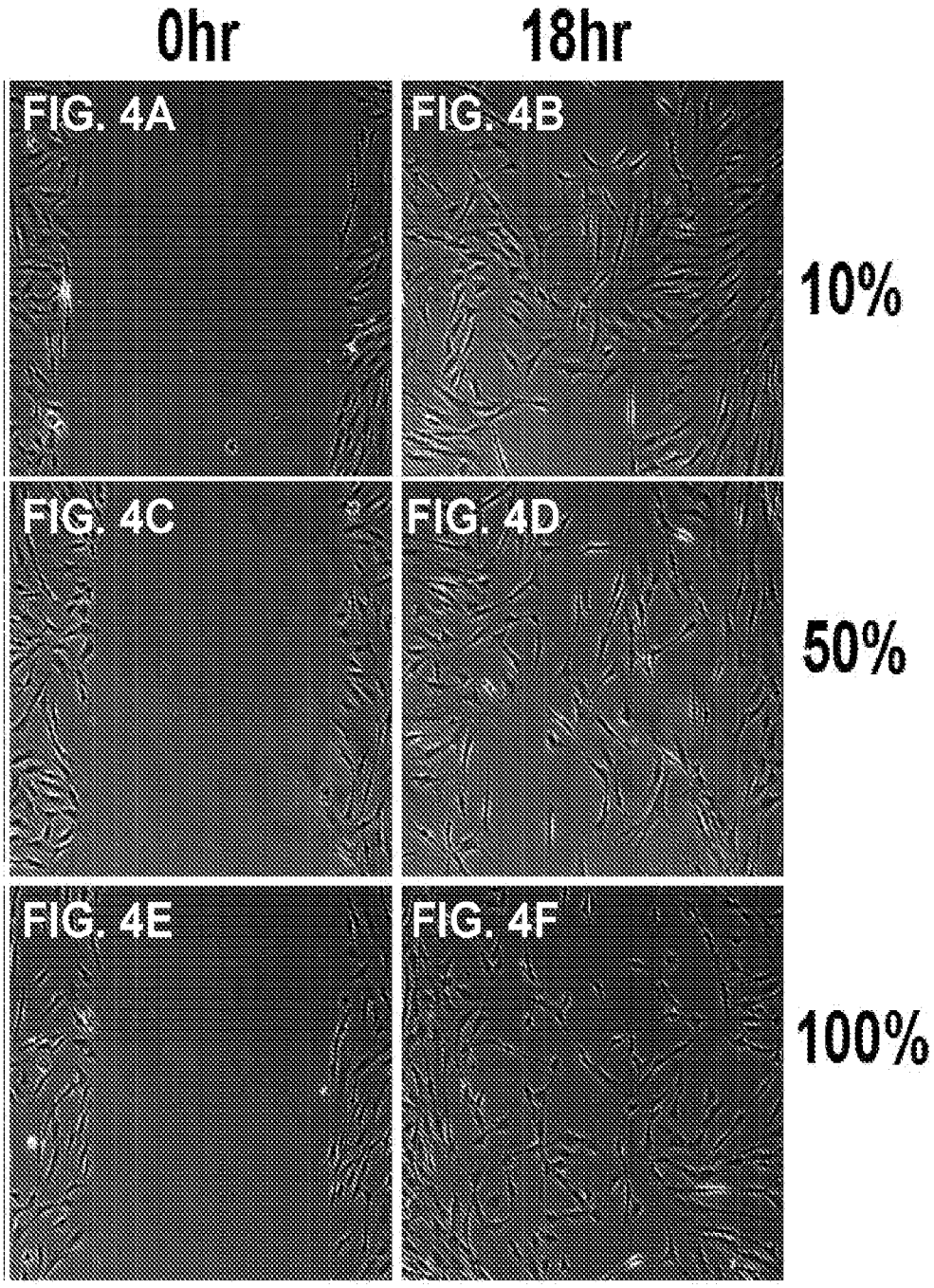


FIG. 3


0hr
18hr
FIG. 4A
FIG. 4B
10%
FIG. 4C
FIG. 4D
50%
FIG. 4E
FIG. 4F
100%
Conditioned
Medium (CM)

FIGS. 4A-4F

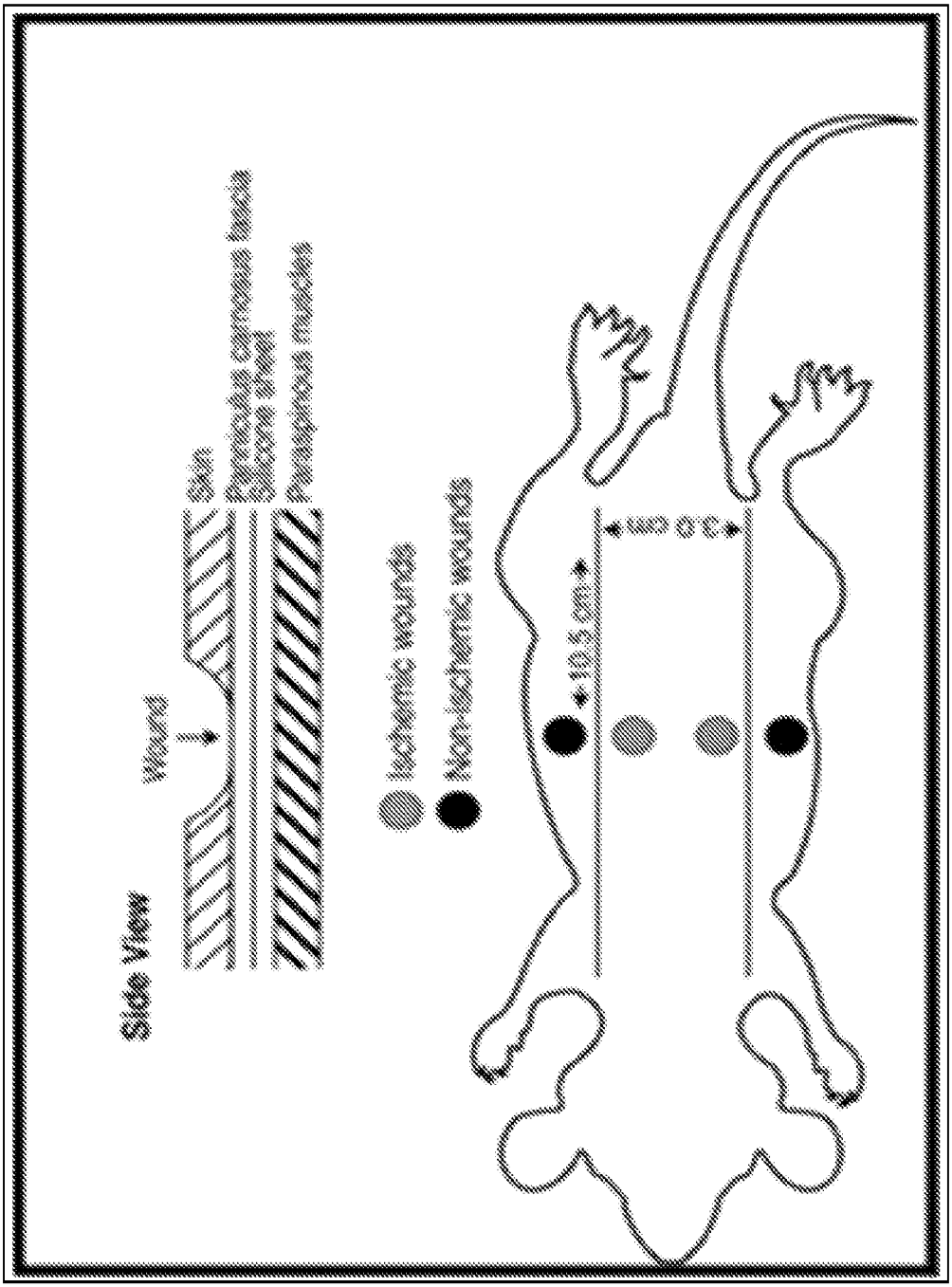

Side View
Wound
Skin
Connective tissue
Silicone film
Panniculus muscle
Ischemic wounds
Non-ischemic wounds
10.5 cm
3 cm

FIG. 5

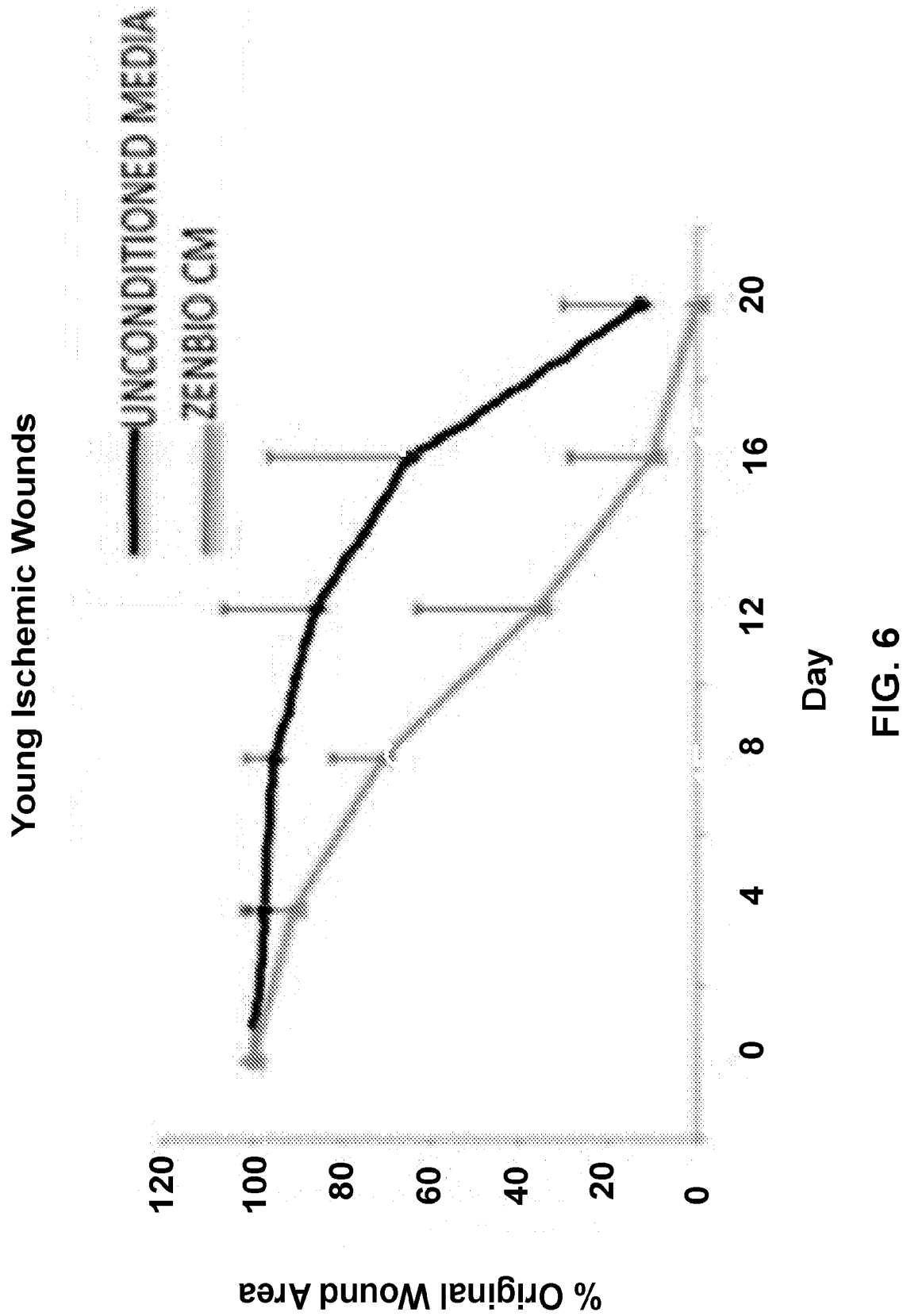

Young Ischemic Wounds
UNCONDITIONED MEDIA
ZENBIO CM
% Original Wound Area
120
100
80
60
40
20
0
0
4
8
12
16
20
Day

FIG. 6

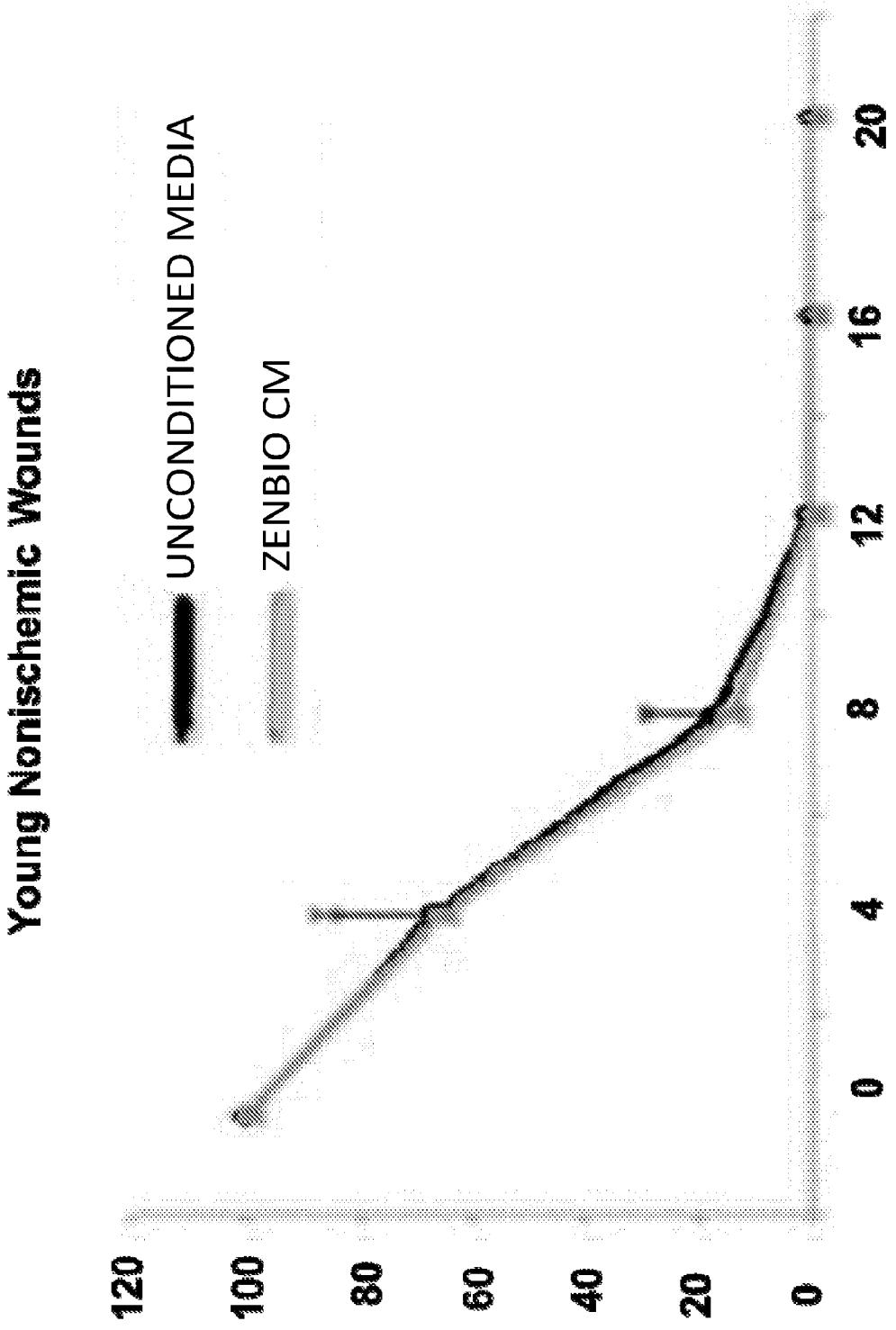

Young Nonischemic Wounds
UNCONDITIONED MEDIA
ZENBIO CM
120
100
80
60
40
20
0
0
4
8
12
16
20
Day

FIG. 7

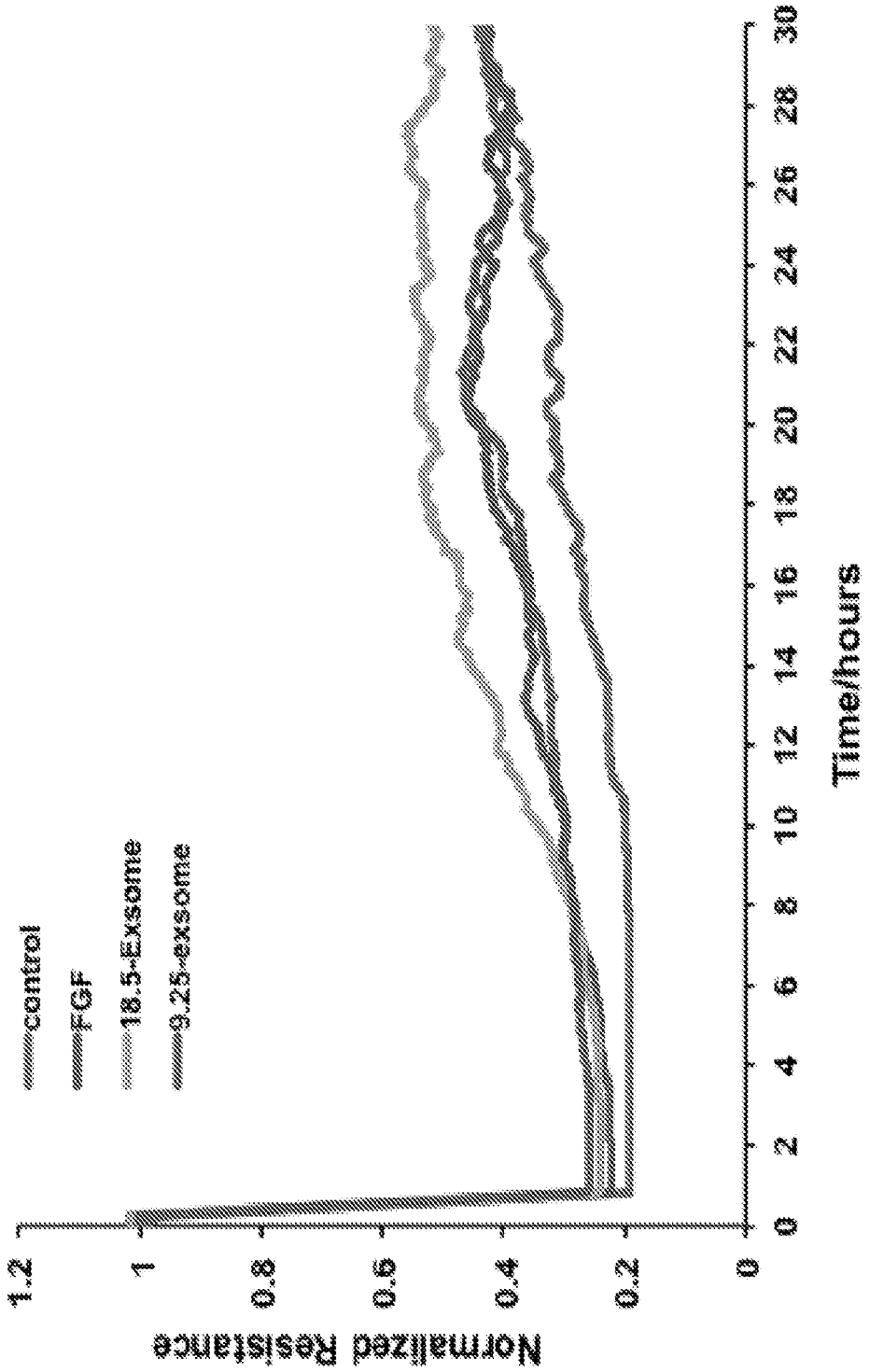

control
FGF
18.5-Exsome
9.25-exsome
Normalized Resistance
1.2
1
0.8
0.6
0.4
0.2
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Time/hours

FIG. 8

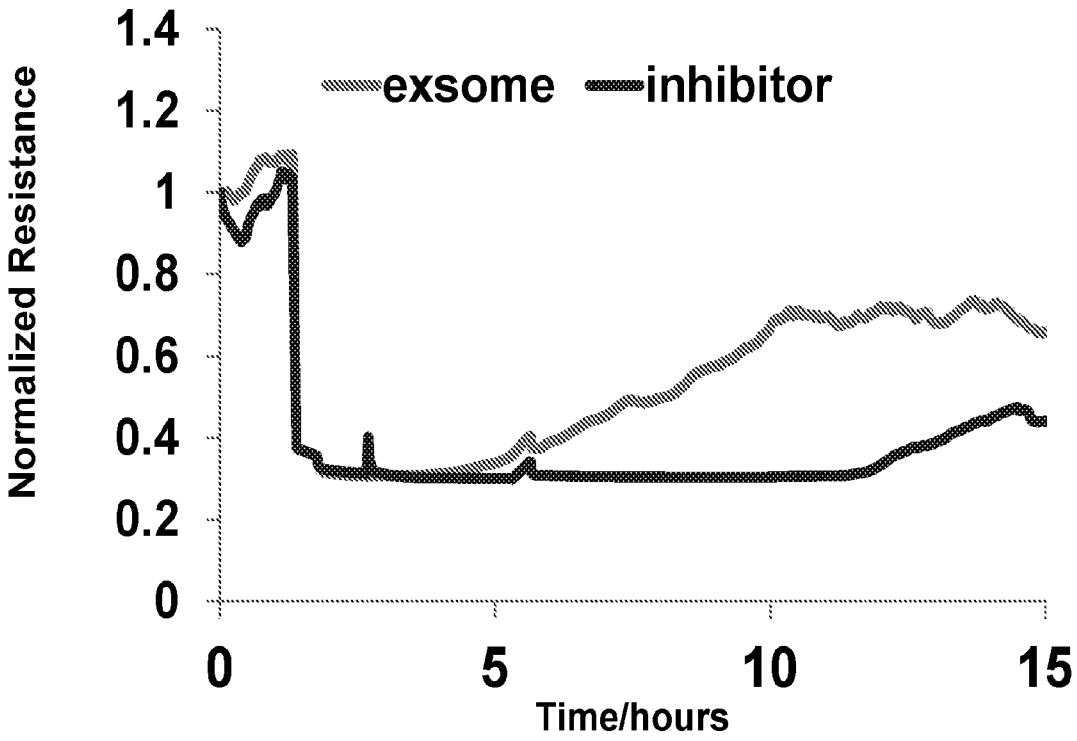

1.4
1.2
1
0.8
0.6
0.4
0.2
0
Normalized Resistance
exsome
inhibitor
0
5
10
15
Time/hours

FIG. 9

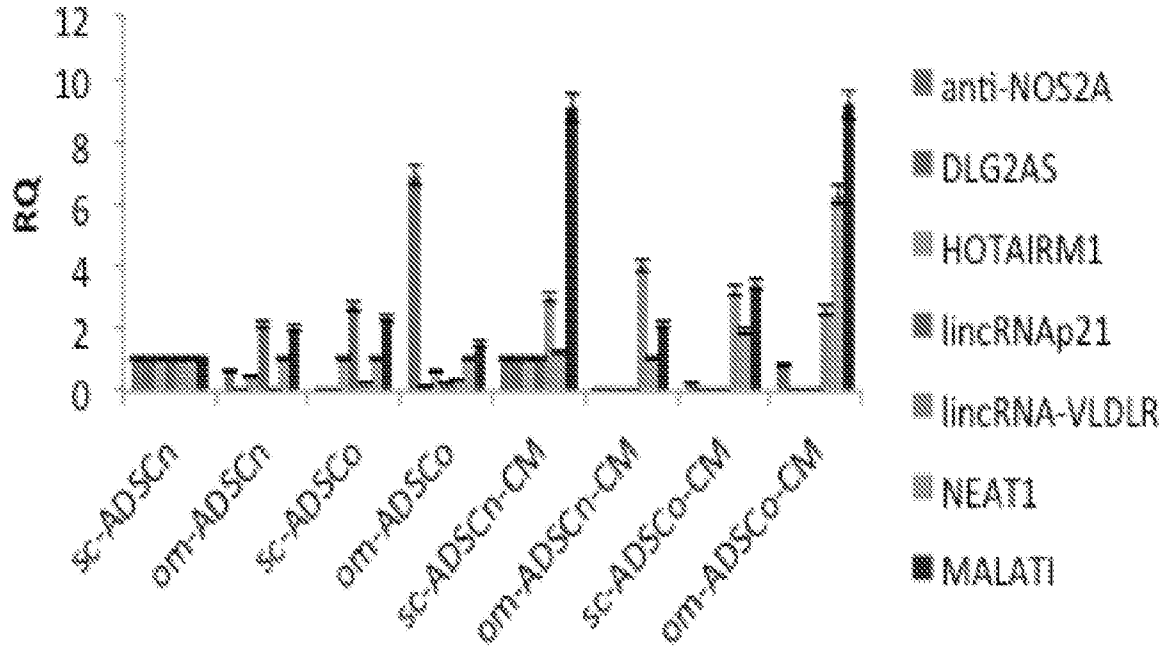


FIG. 10A

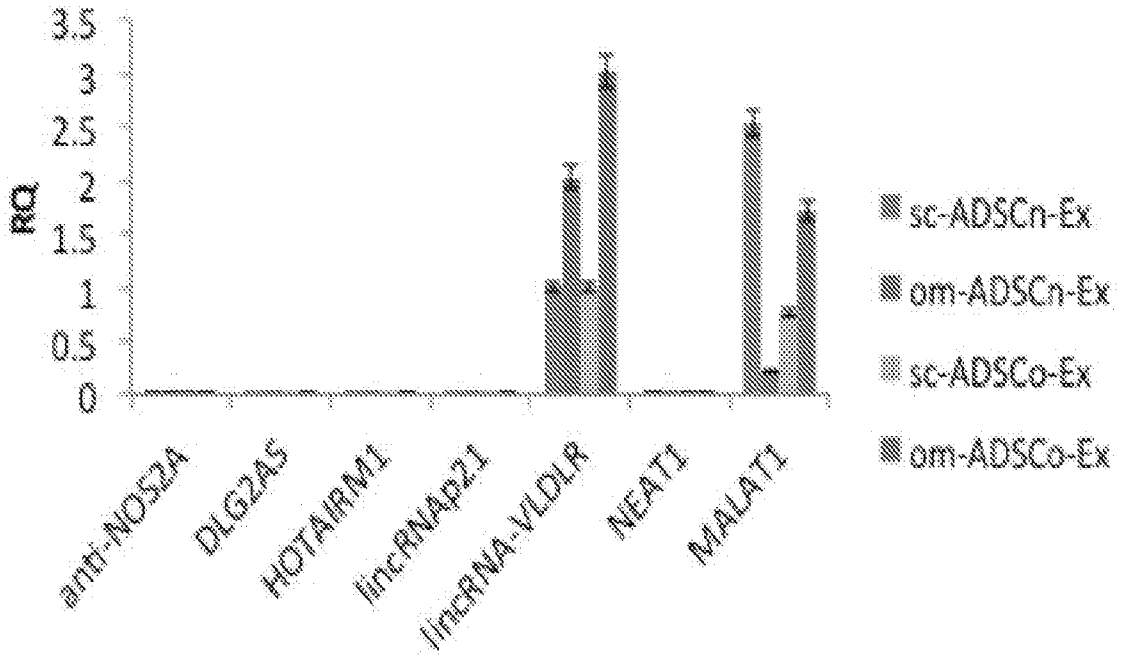
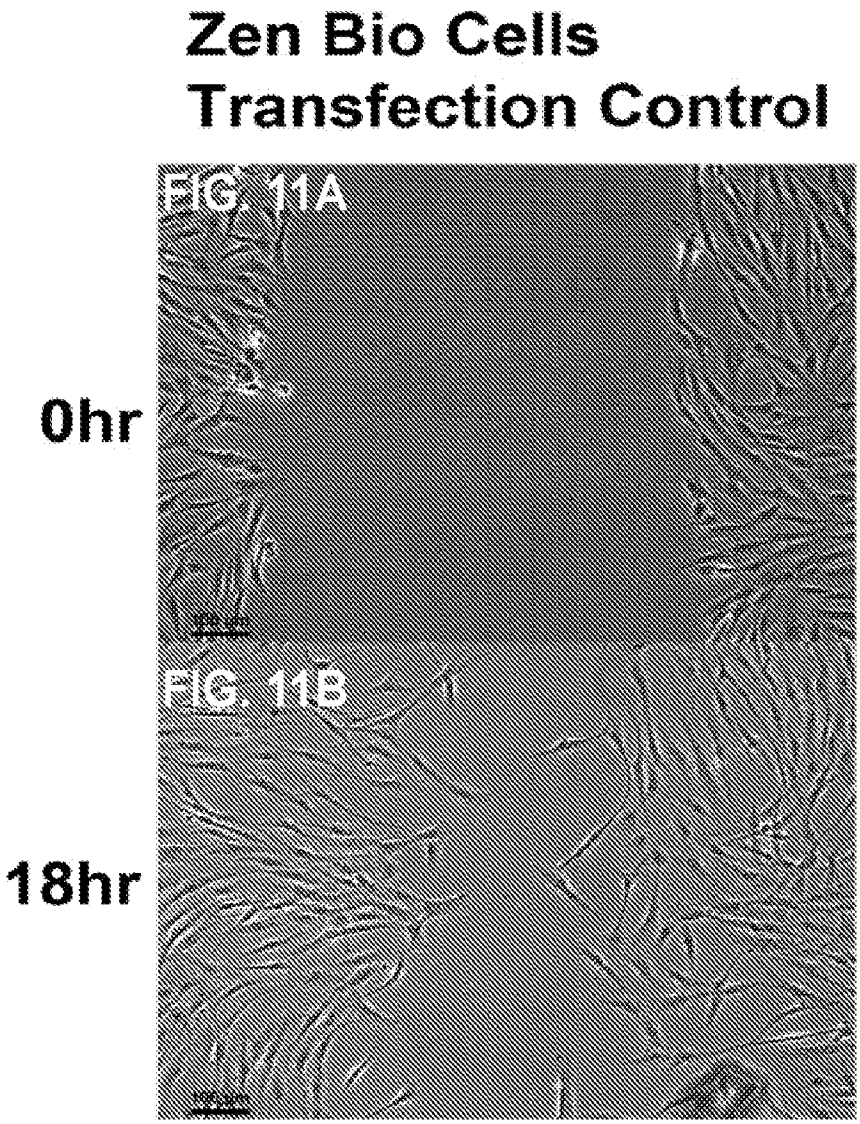


FIG. 10B



FIGS. 11A-11B

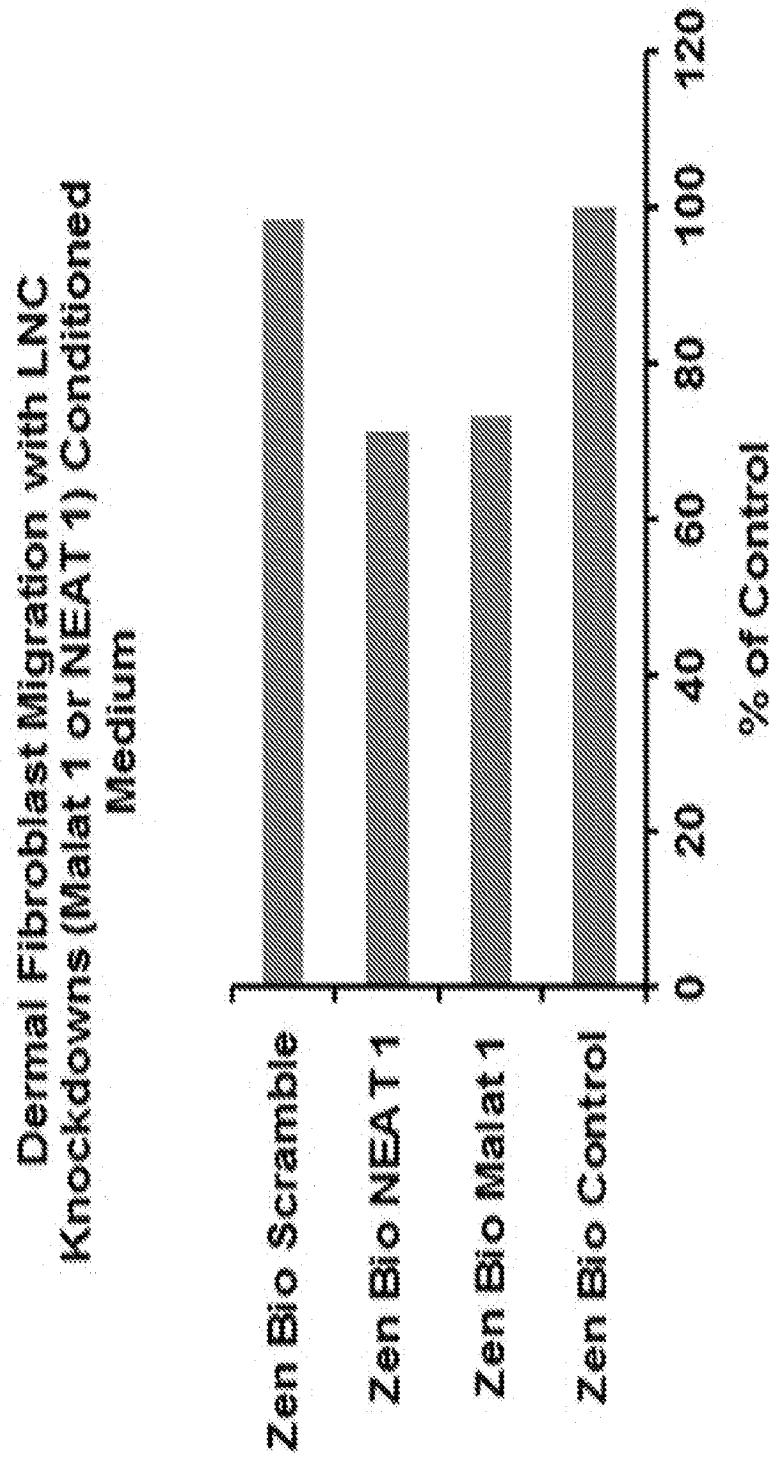
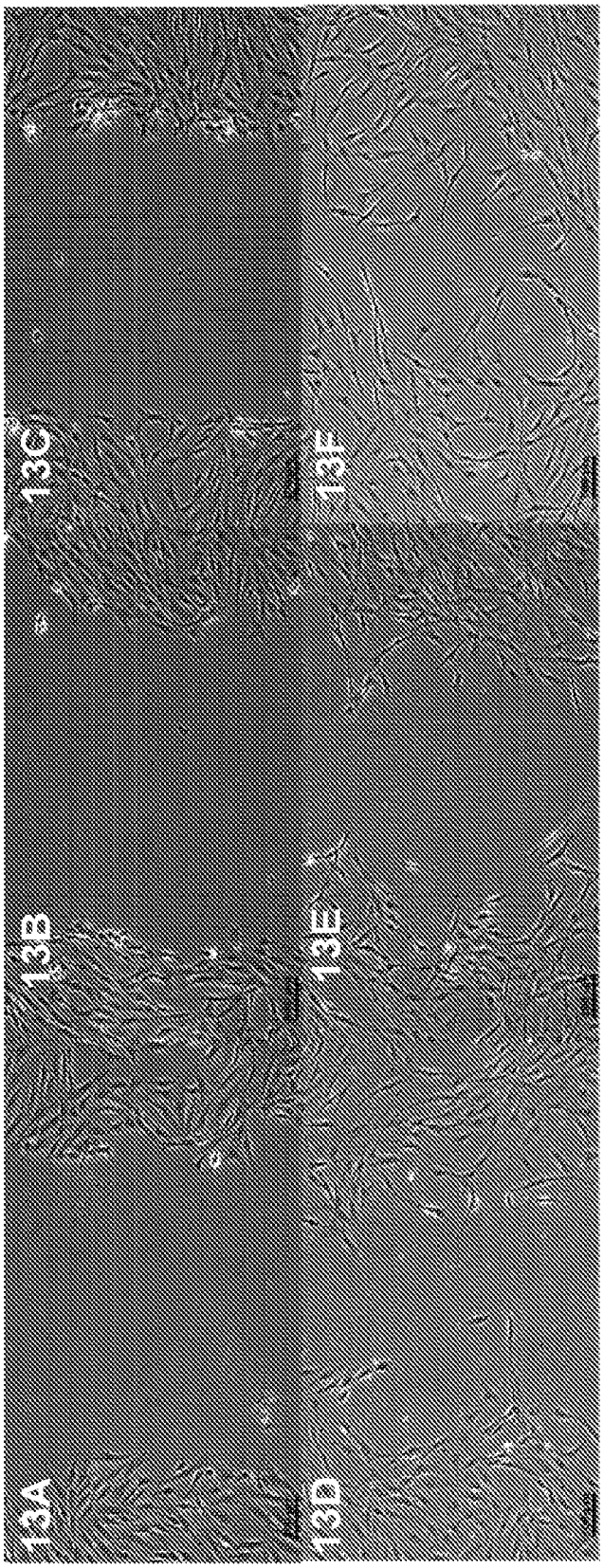

Dermal Fibroblast Migration with LNC Knockdowns (Malat 1 or NEAT 1) Conditioned Medium
Zen Bio Scramble
Zen Bio NEAT 1
Zen Bio Malat 1
Zen Bio Control
0
20
40
60
80
100
120
% of Control

FIG. 12


Knockdown Malat 1
Knockdown NEAT 1
Scramble
13A
13B
13C
13D
13E
13F

FIGS. 13A-13F

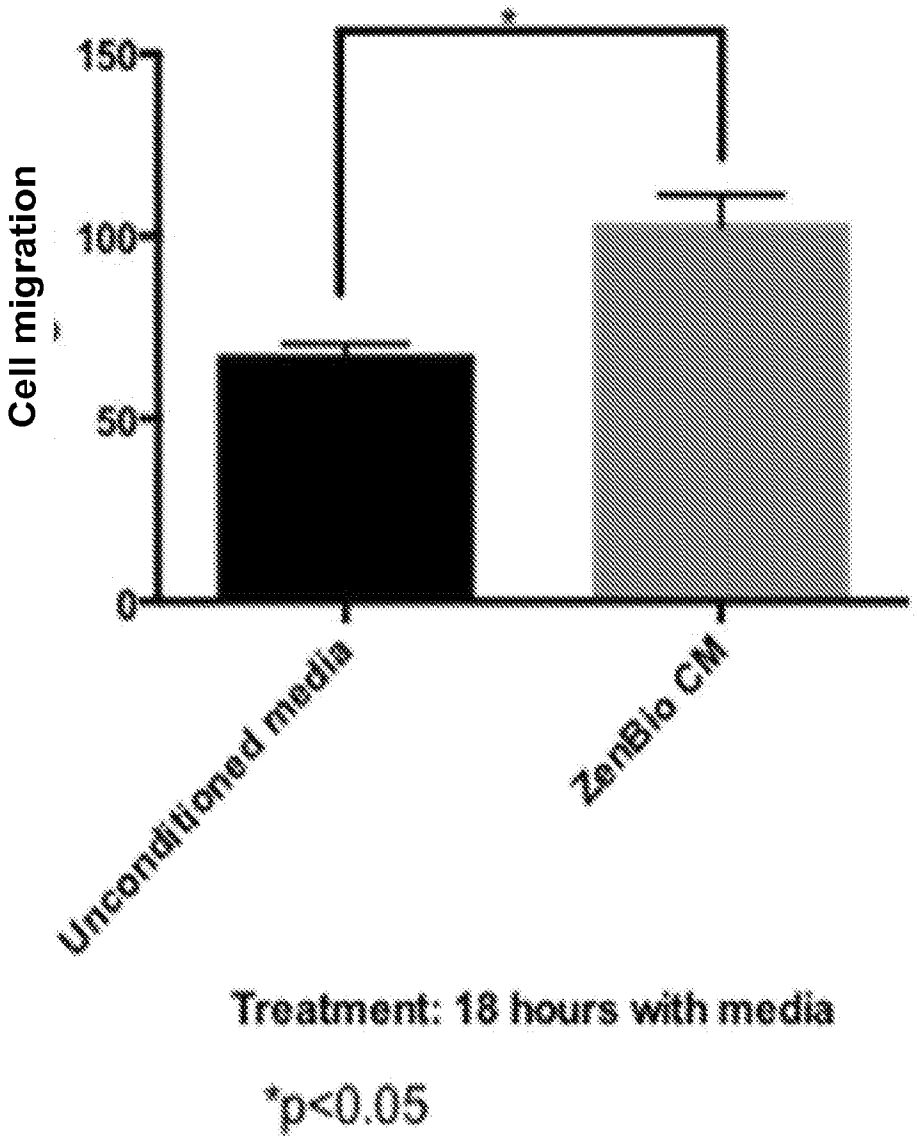

*
150
100
50
0
Cell migration
Unconditioned media
ZenBio CM
Treatment: 18 hours with media
*p<0.05

FIG. 14

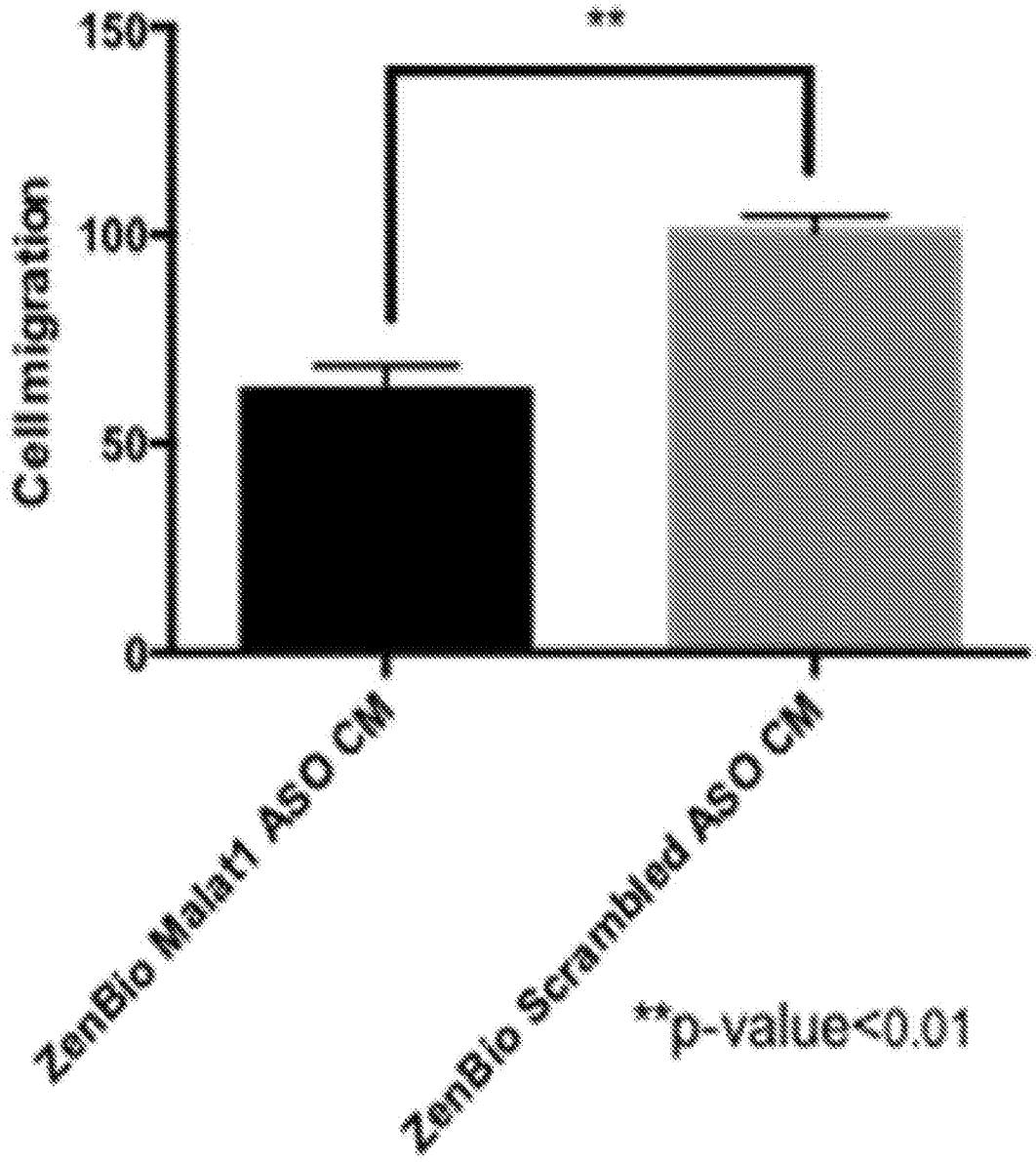

**
150
100
50
0
Cell migration
ZenBio Malat1 ASO CM
ZenBio Scrambled ASO CM
**p-value<0.01

FIG. 15

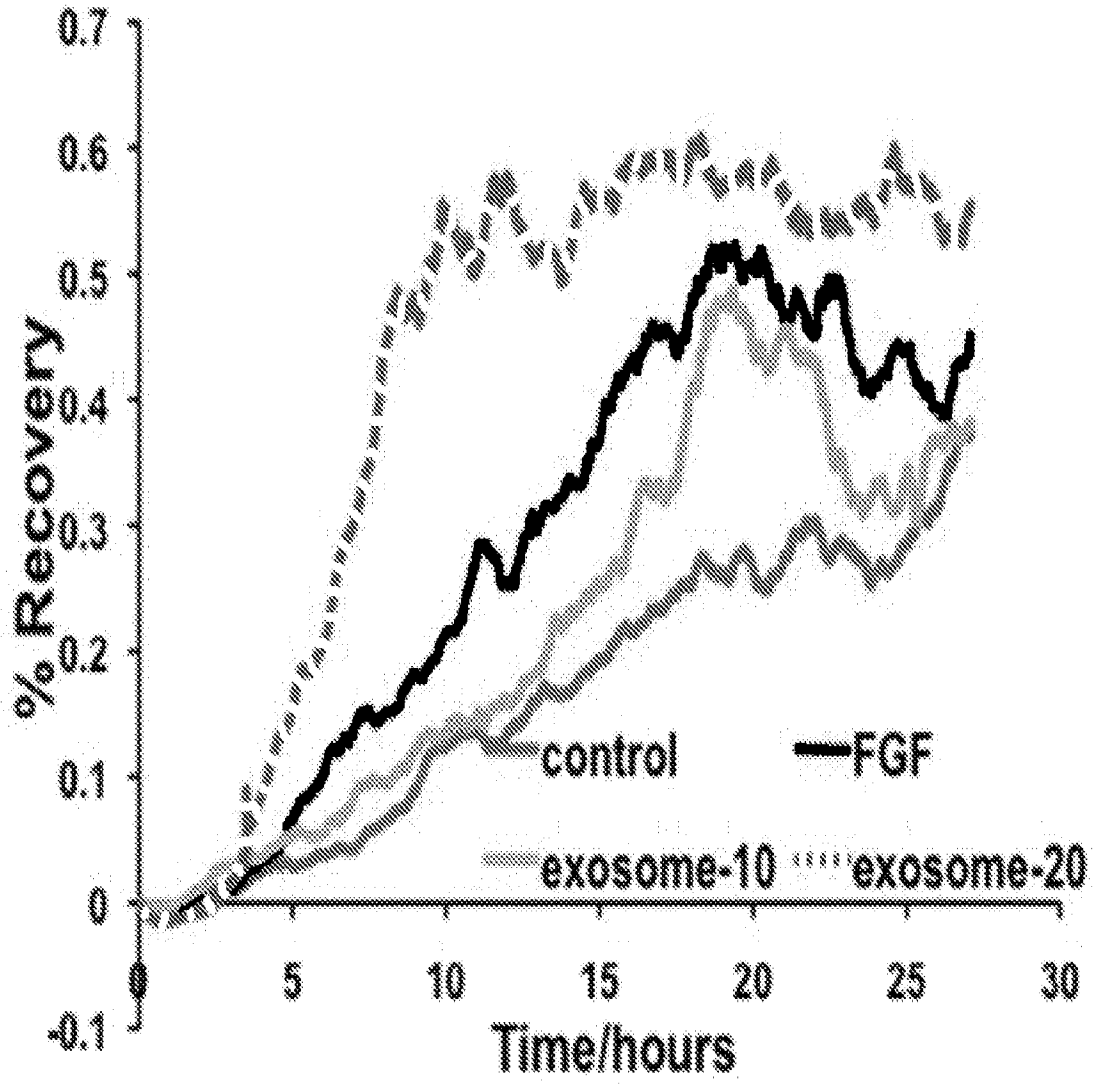

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
% Recovery
0
5
10
15
20
25
30
Time/hours
control
FGF
exosome-10
exosome-20

FIG. 16

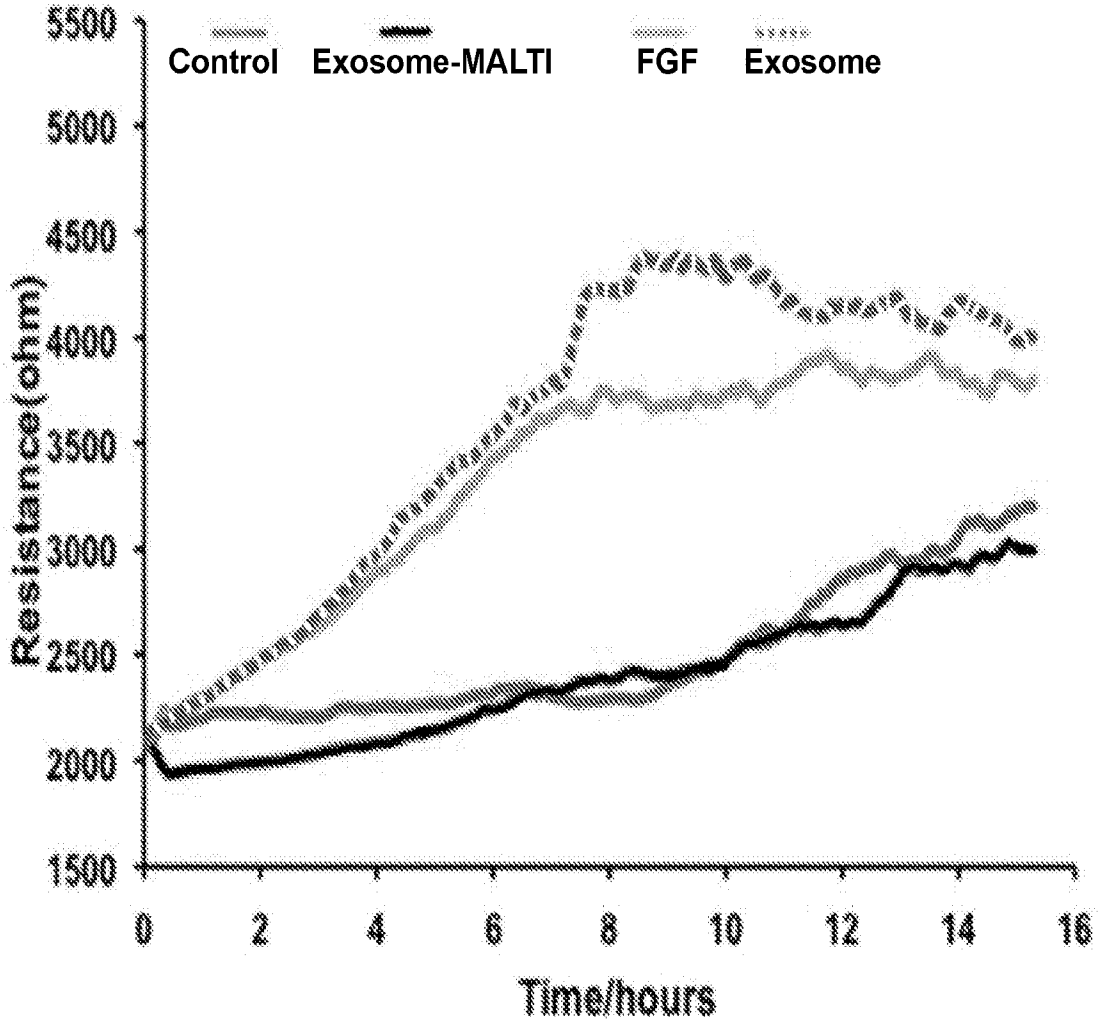

Control
Exosome-MALTI
FGF
Exosome
Resistance(ohm)
5500
5000
4500
4000
3500
3000
2500
2000
1500
0
2
4
6
8
10
12
14
16
Time/hours

FIG. 17

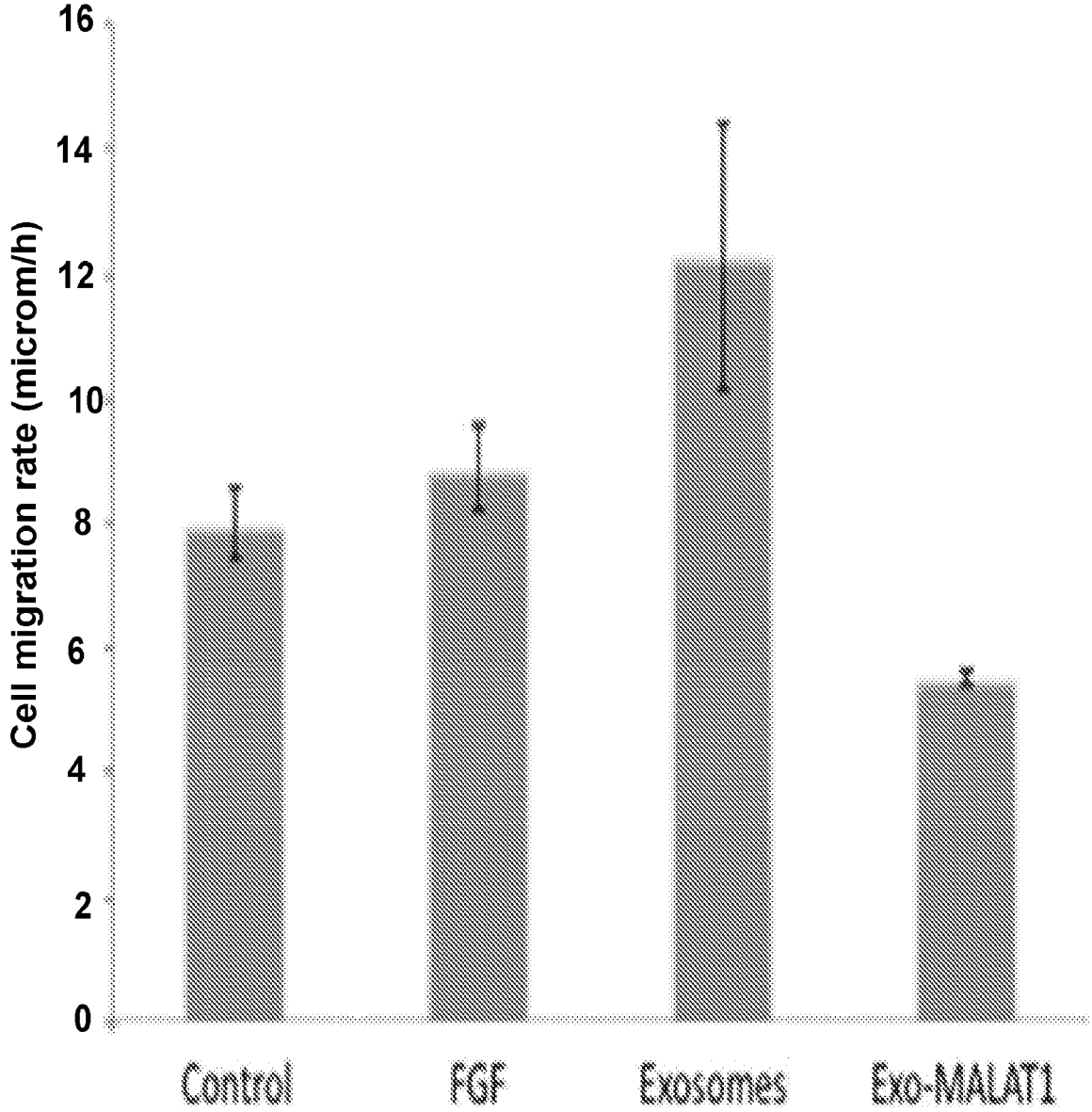

16
14
12
10
8
6
4
2
0
Cell migration rate (microm/h)
Control
FGF
Exosomes
Exo-MALAT1

FIG. 18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/049359

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 35/35; C12N 5/00; C12N 15/113 (2017.01)
CPC - A61K 9/5068; A61K 35/35; C12N 5/0667; C12N 15/113 (2017.08)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/455; 435/458; 435/91.3 (keyword delimited)

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014/0147454 A1 (MODERNATX INC) 29 May 2014 (29.05.2014) entire document	5-8
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Y		1-4, 9
Y	WO 2016/089732 A2 (UNIVERSITY OF SOUTH FLORIDA et al) 09 June 2016 (09.06.2016) entire document	1-4
Y	US 2014/0371296 A1 (ISIS PHARMACEUTICALS INC) 18 December 2014 (18.12.2014) entire document	9
A	US 2016/0041153 A1 (BROWN et al) 11 February 2016 (11.02.2016) entire document	1-9
A	WO 2016072821 A1 (INDUSTRY-UNIVERSITY COOPERATION FOUNDATION - HANYANG UNIVERSITY) 12 May 2016 (12.05.2016) entire document	1-9
A	PATEL et al. "Adipose-derived stem cells from lean and obese humans show depot specific differences in their stem cell markers, exosome contents and senescence: role of protein kinase C delta (PKCδ) in adipose stem cell niche," Stem Cell Investigation, 31 January 2016 (31.01.2016), Vol. 3, No. 2, Pgs. 1-12. entire document	1-9

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
26 October 2017	16 NOV 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/049359

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs:1-3 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/049359

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 10-14
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.