

Description

Title of Invention: ENGINEERED EXOSOMES FOR IMPROVING WOUND HEALING

Field of the Invention

- [0001] The present disclosure relates to engineered exosomes, in particular to an exosome carrying different fusion proteins anchored external to the membrane of the exosome. The present disclosure also relates to a composition comprising the engineered exosome, a nucleic acid construct comprising the polynucleotide encoding the fusion proteins and uses of the engineered exosome in wound healing.

Background

- [0002] Wound healing is an extremely complex process that relies on the interaction of many cell types and mediators in a tightly regulated temporal and spatial sequence. The interaction of platelets, macrophages, fibroblasts, epithelial cells, and endothelial cells with the extracellular matrix, growth inhibiting factors, cytokines, and chemokines occurs in a highly synchronized manner throughout four phases: hemostasis, inflammation, proliferation/migration, and tissue remodeling.
- [0003] Hemostasis occurs immediately after an injury. As the mechanism which leads to the cessation of bleeding from a damaged blood vessel, hemostasis involves multiple interlinked steps. In the initial stage of wound healing, vasoconstriction, platelet activation, and aggregation occur. During hemostasis, platelets release several signaling molecules, including cyclic AMP (cAMP), transforming growth factor- β (TGF- β), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF). The role of platelets in hemostasis is crucial. Platelets comprise the majority of the initial clot and also release signaling molecules responsible for the activation and recruitment of fibroblasts, macrophages, and endothelial cells. PDGF is required for the activation and recruitment of fibroblasts which migrate to the wound site to begin the production of collagen and glycosaminoglycans.
- [0004] The next stage of wound healing is inflammation, which occurs within the first 24 hours after skin damage. The inflammatory phase is characterized by the generation of a highly inflammatory environment through the secretion of several compounds, including pro-inflammatory cytokines, proteases, reactive oxygen species and growth factors. Macrophages play a crucial role during the inflammatory phase, as they are attracted to the wounded area and remove bacteria and dead tissue. As important as their phagocytic role is, macrophages also mediate the transition to the next phase in wound healing. Activated macrophages release soluble mediators that initiate an-

giogenic processes and recruit and activate fibroblasts which will synthesize the new tissue matrix.

[0005] Once the bleeding and inflammation have been controlled, the proliferative phase begins. During the proliferative phase of wound healing, soluble mediators released during the inflammatory phase promote migration of epithelial cells and fibroblasts to the damaged region, thereby supporting capillary growth and new tissue synthesis. Fibroblasts are the key cells in the proliferative phase of wound healing. At this stage, fibroblasts settle down and begin to proliferate and synthesize collagen, fibronectin, proteoglycans, and glycosaminoglycans, which are the major constituents of the extracellular matrix (ECM). Fibroblasts not only produce ECM, but, together with macrophages, vascular endothelial cells, and epidermal cells, also secrete angiogenic signals. As a result of both activities, a transitional replacement for normal dermis called granulation tissue is formed. The newly synthesized, highly vascularized, tissue has significantly less tensile strength than unwounded tissue.

[0006] Finally, in the final phase of wound healing, the granulation tissue is remodeled until its vascularization degree, matrix composition, and tissue tensile strength resemble those of healthy tissue. However, it has been reported that tensile strength is normally increased to a maximum of about 80% of normal tissue.

[0007] Acute wounds normally heal efficiently as they progress throughout the four overlapping phases described above. However, the skin's natural ability to self-regenerate after injury can be seriously compromised under certain circumstances. For instance, altered wound healing capacity has been reported in patients suffering from deep burns, massive skin loss, non-healing ulcers, surgical interventions, and prolonged immobilization. Altered wound healing capacity has also been reported in patients suffering from conditions such as diabetes, neuropathy, and/or vascular disease. In such patients, the healing process is uncoordinated, incomplete, and prolonged, which results in a poor functional outcome. As a result of an inadequate healing process with altered inflammatory, proliferative, and/or remodeling phases, skin lesions can enter into a chronic state. Chronic wounds are characterized by the disruption of the normal regeneration process, with increased risk of infection and complicated, slow healing.

[0008] In an effort to unveil the processes leading to abnormal wound healing, several studies have found important molecular differences in patients suffering from chronic wounds. Remarkably, both the molecular environment and the capacity of the wound cells to respond to cytokines and growth factors is altered in chronic wounds. Further, an increasing number of studies have shown that fibroblasts from non-healing skin ulcers may not be capable of responding to growth factors and divide as fibroblasts in healing wounds.

- [0009] As multiple studies have shown that fibroblasts from chronic wounds display abnormal phenotypes, fibroblasts-focused therapies constitute an area of increased research interest. Current approaches include delivering viable fibroblasts directly to the wound site or the application of growth factors (e.g., PDGF, TGF- β to stimulate fibroblast division or activity.
- [0010] With the increased prevalence of diseases such as diabetes, vascular disease, and obesity, chronic wounds are becoming a major global issue with limited treatment strategies, unsatisfactory therapeutic effects and significant medical costs. Chronic wounds are particularly common in the elderly, with up to 3% of the population over the age of 65 being affected. Thus, chronic wounds not only represent a painful condition profoundly affecting patients' mobility and quality of life, but also a substantial economic burden on the healthcare system.
- [0011] In view of the above limitations, it is desirable to provide methods and compositions that would improve wound healing in a simple, cost-effective and non-invasive manner. This is particularly true when it comes to promoting wound healing following surgical incisions, radiotherapy-induced injuries, diabetic foot ulcers, burns, and/or other acute or chronic wounds.
- [0012] Accordingly, methods for improving wound healing are desirable, including, but not limited to, methods for healing wounds such as deep burns, massive skin loss, non-healing ulcers, surgical interventions, radiotherapy-induced injuries, and/or other acute or chronic wounds.

Summary of the Invention

- [0013] In one aspect of the invention, provided is an engineered exosome, comprising (a) an EGF polypeptide, fused to a first anchoring polypeptide, (b) a NGF polypeptide, fused to a second anchoring polypeptide, and (c) a PDGF-BB polypeptide, fused to a third anchoring polypeptide, wherein (a), (b) and (c) are anchored on a membrane of the exosome via the first, second and third anchoring polypeptide, respectively, and wherein the EGF polypeptide, the NGF polypeptide and the PDGF-BB polypeptide are exposed on an outer surface of the membrane of the exosome.
- [0014] In some embodiments, the engineered exosome further comprises (d) an IDR-1018 polypeptide comprising the amino acid sequence shown in SEQ ID NO. 4, fused to a fourth anchoring polypeptide, and the IDR-1018 polypeptide is exposed on the outer surface of the membrane of the exosome.
- [0015] In some embodiments, the EGF polypeptide is a human EGF polypeptide or an ortholog or paralog thereof, preferably the EGF polypeptide comprises an amino acid sequence having at least 80% identity to the amino acid sequence as shown in SEQ ID NO. 1.

- [0016] In some embodiments, the NGF polypeptide is a human NGF polypeptide or an ortholog or paralog thereof, preferably the NGF polypeptide comprises an amino acid sequence having at least 80% identity to the amino acid sequence as shown in SEQ ID NO. 2.
- [0017] In some embodiments, the PDGF-BB polypeptide is human PDGF-BB dimer or an ortholog or paralog thereof; more preferably the PDGF-BB dimer comprises an amino acid sequence having at least 80% identity to the amino acid sequence as shown in SEQ ID NO. 3.
- [0018] In some embodiments, the anchoring polypeptides are membrane proteins of exosome, membrane-targeting sequences, or an anchoring functional fragment thereof. Exemplary membrane proteins of exosome include but are not limited to lamp2b, tetraspanins such as CD63, CD9 and CD81, platelet-derived growth factor receptors (PDGFRs), lactadherin (C1C2 domain), vesicular stomatitis virus glycoprotein (VSVG), prostaglandin F2 receptor negative regulator (PTGFRN) and any combination thereof. Exemplary membrane-targeting sequences include but are not limited to glycosylphosphatidylinositol (GPI) anchors and lipid-anchored proteins.
- [0019] In preferable embodiments, the anchoring polypeptides comprise a full-length CD63 or a truncated CD63 that retains TM3 domain. In preferable embodiments, each of the first, second, third, and fourth, when present, anchoring polypeptides comprises a TM3 domain of CD63. In preferable embodiments, each of the first, second, third, and fourth, when present, anchoring polypeptides is a TM3 domain of CD63. In some embodiments, each of the anchoring polypeptides is a TM3 domain of CD63; preferably, the TM3 domain of CD63 comprises an amino acid sequence having at least 80% identity to the amino acid sequence as shown in SEQ ID NO. 9.
- [0020] In some embodiments, the EGF polypeptide, the NGF polypeptide, the PDGF-BB polypeptide, and the IDR-1018 polypeptide, when present, is fused to the N or C-terminus of respective anchoring polypeptide, optionally through a peptide linker, preferably, the peptide linker consists of glycine and serine, e.g., (G4S)_n, in which n is an integer from 1 to 3.
- [0021] In some embodiments, the exosome is not derived from a mesenchymal stem cell; preferably, the exosome is not derived from a stem cell.
- [0022] Another aspect of the disclosure relates to a composition comprising the exosome as described herein, and a carrier; preferably, the composition is a liquid formulation; more preferably, the composition is formulated for topical or intradermal administration; more preferably, the composition is a pharmaceutical composition.
- [0023] In some embodiments, the composition does not contain an EGF polypeptide (e.g. human EGF polypeptide), a NGF polypeptide (e.g., human NGF polypeptide), a

PDGF-BB polypeptide (e.g., a human PDGF-BB polypeptide) , or an IDR-1018 polypeptide, not attached to the membrane of the exosome.

- [0024] Another aspect of the disclosure relates to a nucleic acid construct comprising a polynucleotide encoding (a) an EGF polypeptide fused to a first anchoring polypeptide, (b) a NGF polypeptide fused to a second anchoring polypeptide, and (c) a PDGF-BB polypeptide, fused to a third anchoring polypeptide.
- [0025] In some embodiments, the nucleic acid construct comprising a polynucleotide further encoding (d) an IDR-1018 polypeptide comprising the amino acid sequence shown in SEQ ID NO. 4, fused to a fourth anchoring polypeptide.
- [0026] In some embodiments, the EGF polypeptide is a human EGF polypeptide or an ortholog or paralog thereof, preferably the EGF polypeptide comprises an amino acid sequence having at least 80%identity to the amino acid sequence as shown in SEQ ID NO. 1.
- [0027] In some embodiments, the NGF polypeptide is a human NGF polypeptide or an ortholog or paralog thereof, preferably the NGF polypeptide comprises an amino acid sequence having at least 80%identity to the amino acid sequence as shown in SEQ ID NO. 2.
- [0028] In some embodiments, the PDGF-BB polypeptide is a human PDGF-BB dimer; more preferably the PGDF-BB dimer comprises an amino acid sequence having at least 80%identity to the amino acid sequence as shown in SEQ ID NO. 3.
- [0029] In some embodiments, a polynucleotide encoding the EGF polypeptide has a nucleotide sequence as shown in SEQ ID NO. 5 or a degenerate sequence thereof.
- [0030] In some embodiments, a polynucleotide encoding the NGF polypeptide has a nucleotide sequence as shown in SEQ ID NO. 6 or a degenerate sequence thereof.
- [0031] In some embodiments, a polynucleotide encoding the PDGF-BB polypeptide has a nucleotide sequence as shown in SEQ ID NO. 7 or a degenerate sequence thereof.
- [0032] In some embodiments, a polynucleotide encoding the IDR-1018 polypeptide has a nucleotide sequence as shown in SEQ ID NO. 8 or a degenerate sequence thereof.
- [0033] In some embodiments, the anchoring polypeptides are membrane proteins of exosome, membrane-targeting sequences, or an anchoring functional fragment thereof. Exemplary membrane proteins of exosome include but are not limited to lamp2b, tetraspanins such as CD63, CD9 and CD81, platelet-derived growth factor receptors (PDGFRs) , lactadherin (C1C2 domain) , vesicular stomatitis virus glycoprotein (VSVG) , prostaglandin F2 receptor negative regulator (PTGFRN) and any combination thereof. Exemplary membrane-targeting sequences include but are not limited to glycosylphosphatidylinositol (GPI) anchors and lipid-anchored proteins.
- [0034] In preferable embodiments, the anchoring polypeptides comprise a full-length CD63 or a truncated CD63 that retains TM3 domain. In preferable embodiments, each of

the first, second, third, and fourth, when present, anchoring polypeptides comprises a TM3 domain of CD63. In preferable embodiments, each of the first, second, third, and fourth, when present, anchoring polypeptides is a TM3 domain of CD63. In some embodiments, a polynucleotide encoding the TM3 domain of CD63 has a nucleotide sequence as shown in SEQ ID NO. 10 or a degenerate sequence thereof.

- [0035] In some embodiments, the polynucleotide is a single polynucleotide comprising a fragment of nucleotides encoding the polypeptides (a) , (b) , (c) and, optionally, (d) , with each of the polypeptides (a) , (b) , (c) and, optionally, (d) linked to the anchoring polypeptide; preferably the polypeptides (a) , (b) , (c) and, optionally, (d) are spaced by self-cleavage peptides; optionally, the self-cleavage peptides are 2A peptides, e.g., T2A, E2A, P2A or any combination thereof.
- [0036] Another aspect of the disclosure relates to a vector comprising the nucleic acid construct as described in any of the nucleic acid construct described herein.
- [0037] Another aspect of the disclosure relates to a cell transduced with the vector as described herein, wherein the polynucleotide is integrated into the genome of the cell.
- [0038] In some embodiments, the cell is not a mesenchymal stem cell; preferably, the cell is not a stem cell; preferably, the cell is a mammalian cell; or more preferably, the cell is a HEK293 or CHO cell.
- [0039] Another aspect of the disclosure relates to a method of producing an engineered exosome as disclosed herein, comprising (a) transducing the cell as described above with the vector as described above; (b) culturing the cell in a condition allowing secretion of an exosome from the cell; and (c) collecting and purifying the exosome.
- [0040] In some embodiments, the method further comprises adapting the cell to a serum-free condition during step (b) .
- [0041] A further aspect of the disclosure relates to use of the engineered exosomes, or the composition as disclosed herein in manufacturing a medicament for improving wound healing or for treating wound; preferably, the wound is a chronic wound, preferably a diabetic wound; more preferably, the wound includes diabetic foot ulcer.
- [0042] A further aspect of the disclosure relates to a method for improving wound healing or for treating wound; preferably, the wound is a chronic wound, preferably a diabetic wound; more preferably, the wound includes diabetic foot ulcer.
- [0043] A further aspect of the disclosure relates to the engineered exosomes, or the composition as disclosed herein for use in improving wound healing or treating wound; preferably, the wound is a chronic wound, preferably a diabetic wound; more preferably, the wound includes diabetic foot ulcer.
- [0044] A further aspect of the disclosure relates to the use of the engineered exosomes, or the composition as disclosed herein in manufacturing a medicament for inhibiting *Cutibacterium acnes* (formerly known as *Propionibacterium acnes*) infection, e.g.,

in skin; preferably, the Cutibacterium acnes infection is acnes or acnes skin such as a scar or cutaneous damage caused by acnes. A further aspect of the disclosure relates to a method for treating Cutibacterium acnes infection, e.g., in skin; preferably, the Cutibacterium acnes infection is acnes or acnes skin such as a scar or cutaneous damage caused by acnes. A further aspect of the disclosure relates to the engineered exosomes, or the composition as disclosed herein for use in treating Cutibacterium acnes infection, e.g., in skin; preferably, the Cutibacterium acnes infection is acnes or acnes skin such as a scar or cutaneous damage caused by acnes.

[0045] These and other aspects and advantages of the disclosure will be apparent from the detailed description provided in the following.

Brief Description of the Figures

[0046] Figure 1. Construction of stable cell lines, which secrete engineered exosomes loading with functional proteins. Mammalian cells, e.g. HEK293 cells were cultured and infected with lentiviruses, which contain functional genes, EGF, NGF, PDGF-BB and IDR-1018. Then antibiotics was used for stable cell line selection. After three passages selection, the expression of the functional genes was identified in both cell pellet and exosomes. After confirmation of the expression, an adaptation of stable cell line to a serum-free condition was performed. Finally, collect the cultured supernatant from the serum-free stable cell line and the identify the exosomes purified via ultra-centrifuge.

[0047] Figure 2. Identification of engineered exosomes derived from the stable cell lines. (A) The particle diameter and particle concentration of exosomes derived from the stable cell lines were analyzed by NanoFCM. (B) The exosomes were further inspected by transmission electron microscopy (TEM) . (C) Immunoblotting analysis of exosomes with antibodies against CD63, the exosomes fused scaffold protein.

[0048] Figure 3. HSF (human skin fibroblast) cells were prepared for scratch wounding assay. Once HSF cells were at confluence, scrape cell layer in a straight line using a 1 mm pipette tip. After scratching, gently wash cell monolayer to remove detached cells, then replenish with fresh medium (Mock) or exosomes (No. 1, No. 37, No. 39) , respectively. Then images were captured using microscope on 10x magnification at different time points (0h, 24h, 48h) .

[0049] Figure 4. Construction of a wound healing model with diabetic foot ulcer (DFU) rat. The Sprague-Dawley rats were used to establish DFU (diabetic foot ulcer) models by streptozotocin and skin biopsy punch. (A) . The schematic diagram of construction of DFU rat model and design of the intradermal administration. The blood glucose (B) and body weight (C) of the animals from different groups (n = 5) within DFU model are shown at different time points. The groups contain the health group (Health-

- Vehicle) , DFU model group (Model-Vehicle) , and DFU model treated with exosome (NO. 38) or (NO. 40) groups. I. P, Intraperitoneal injection; I. D, intradermal injection.
- [0050] Figure 5. Wound healing at different time points after exosomes (NO. 38) or (NO. 40) treatment. The wound healing rate over 13 days for each group (n = 5) including the health group (Health-Vehicle) , DFU model group (Model-Vehicle) , DFU model treated with exosome (NO. 38) or (NO. 40) groups was calculated. Data were shown as mean $\pm$ SD. *represent $p < 0.05$ compared with the control group.
- [0051] Figure 6. Engineered exosomes promoted blood vessel and nerve repair in rat DFU model. The wound tissues of DFU rats were collected at the end of the study. The HE staining within DFU model group (Model-Vehicle) and DFU model treated with exosome (NO. 40) was analyzed. The vessel numbers (A-C) were confirmed with CD31 and the nerve repair was shown with PGP9.5 as a detection marker (D-F) .
- [0052] Figure 7. Normal injury healing at different time points treated with exosomes (NO. 40) . (A) The Normal injury healing rate over 16 days for each group (n = 6) including the model group (Vehicle) and model treated with exosome (NO. 40) groups was calculated. (B-C) At the end of study (Day 16) , the tissue at the injury sites were prepared for HE staining, and the escharosis and inflammatory cells were analyzed. Data were shown as mean $\pm$ SD. *represent $p < 0.05$ compared with the control group.

Detailed Description of the Invention

- [0053] Definition
- [0054] The words “a” and “an” when used in the present specification in concert with the word comprising, including the claims, denote “one or more. ”
- [0055] As used herein, the terms “or” and “and/or” are utilized to describe multiple components in combination or exclusive of one another. For example, “x, y, and/or z” can refer to “x” alone, “y” alone, “z” alone, “x, y, and z, ” “ (x and y) or z, ” “x or (y and z) , ” or “x or y or z. ” It is specifically contemplated that x, y, or z may be specifically excluded from an embodiment.
- [0056] Throughout this application, the term “about” is used according to its plain and ordinary meaning in the area of cell and molecular biology to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.
- [0057] “Homology” or “identity” or “similarity” refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous

positions shared by the sequences. An “unrelated” or “non-homologous” sequence shares less than 40%identity, though preferably less than 25%identity, with one of the sequences of the present disclosure.

- [0058] A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99%) of “sequence identity” to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art.
- [0059] The term “linker” as used herein refers to a short fragment of amino acid (AA) or nucleotide sequence containing two or more amino acids or nucleotides which may be same or different.
- [0060] As used herein, “cell line” refers to a population of cells formed by one or more sub-cultivations of a primary cell culture. Each round of subculturing is referred to as a passage. When cells are subcultured, they are referred to as having been passaged. A specific population of cells, or a cell line, is sometimes referred to or characterized by the number of times it has been passaged. For example, a cultured cell population that has been passaged ten times may be referred to as a P10 culture. The primary culture, i.e., the first culture following the isolation of cells from tissue, is designated P0. Following the first subculture, the cells are described as a secondary culture (P1 or passage 1) . After the second subculture, the cells become a tertiary culture (P2 or passage 2) , and so on. It will be understood by those of skill in the art that there may be many population doublings during the period of passaging; therefore, the number of population doublings of a culture is greater than the passage number. The expansion of cells (e.g., the number of population doublings) during the period between passaging depends on many factors, including but not limited to seeding density, substrate, medium, growth conditions, and time between passaging.
- [0061] The terms “reduce,” “inhibit,” “diminish,” “suppress,” “decrease,” “prevent” and grammatical equivalents (including “lower,” “smaller,” etc.) when in reference to the expression of any symptom in an untreated subject relative to a treated subject, mean that the quantity and/or magnitude of the symptoms in the treated subject is lower than in the untreated subject by any amount that is recognized as clinically relevant by any medically trained personnel. In one embodiment, the quantity and/or magnitude of the symptoms in the treated subject is at least 10%lower than, at least 25%lower than, at least 50%lower than, at least 75%lower than, and/or at least 90%lower than the quantity and/or magnitude of the symptoms in the untreated subject.
- [0062] As used herein, the term “therapeutically effective amount” is synonymous with “effective amount” , “therapeutically effective dose” , and/or “effective dose” and

refers to the amount of compound that will elicit the biological, cosmetic, or clinical response being sought by the practitioner in an individual in need thereof. As one example, an effective amount is the amount sufficient to reduce hair loss. The appropriate effective amount to be administered for a particular application of the disclosed methods can be determined by those skilled in the art, using the guidance provided herein. For example, an effective amount can be extrapolated from in vitro and in vivo assays as described in the present specification. One skilled in the art will recognize that the condition of the individual can be monitored throughout the course of therapy and that the effective amount of an exosome or composition disclosed herein that is administered can be adjusted accordingly.

- [0063] As used herein, the terms “treatment,” “treat,” or “treating” refers to intervention in an attempt to alter the natural course of the individual or cell being treated and may be performed either for prophylaxis or during the course of pathology of a disease or condition. Treatment may serve to accomplish one or more of various desired outcomes, including, for example, preventing occurrence or recurrence of disease, alleviation of symptoms, and diminishment of any direct or indirect pathological consequences of the disease, lowering the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis.
- [0064] The term “subject,” as used herein, may be used interchangeably with the term “individual” or “patient” and generally refers to an individual in need of a therapy. The subject can be a mammal, such as a human, dog, cat, horse, pig, or rodent.
- [0065] “Carrier”, as used herein, refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic agent is administered. Such pharmaceutical carriers can be sterile liquids, such as saline solutions in water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. A saline solution is a preferred carrier when the composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsions, tablets, pills, capsules, powders, sustained-release formulations, and the like. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

- [0066] The term “N-terminal amino acid residue” or “N-terminus” refers to the first amino acid residue (amino acid number 1) of a polypeptide or peptide. The term “C-terminal amino acid residue” or “C-terminus” refers to the last amino acid residue (amino acid number n, wherein n = the total number of residues in the peptide or polypeptide) of a polypeptide or peptide.
- [0067] The term “EGF” refers to epidermal growth factor, a growth factor that stimulates cell growth, proliferation and differentiation by binding to its receptor EGFR. It has been proven to be a potent mitogen by stimulating mRNA, DNA and protein synthesis of epithelial cells. It is a single-chain polypeptide consisting of 53 amino acids that is derived from the cleavage of a large precursor, prepro-EGF. EGF is now known as the prototype of the group I EGF family that also includes transforming growth factor- α (TGF- α), heparin-binding EGF (HB-EGF), amphiregulin, betacellulin, epiregulin and epigen. Structurally, they all contain one or more EGF repeats (EGF motif) in their extracellular domain, which is a sequence of 35–40 amino acids spaced by six conserved cysteines in the following pattern: CX7CX3–5CX10–12CXCX5GXRC (C, cysteine; G, glycine; R, arginine; X, other amino acids). One glycine and one arginine in this sequence are also conserved in all EGF-related growth factors but not in proteins that contain EGF motifs without growth factor activity. The six cysteines pair and form three intramolecular disulfide bonds with the following interactions: C1–C3, C2–C4 and C5–C6 (numbered according to their order in the sequence), which are important for maintaining their biological activities. Functionally, these growth factors share the ability to bind the same receptor, the EGF receptor (EGFR, ErbB1), activate its intrinsic tyrosine kinase activity, and couple the receptor to downstream signaling pathways controlling cell proliferation, differentiation, survival, or motility. The amino acid sequence of human EGF is available from GenBank: AAS83395.1, which is also shown as SEQ ID NO. 1 in the present disclosure.
- [0068] The term “NGF” refers to nerve growth factor (NGF) which is the first discovered member of a family of neurotrophic factors, collectively indicated as neurotrophins, which include brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin 4/5. These factors share significant structural homologies and derive from a common ancestor gene. Studies have demonstrated the protective action of NGF not only in the survival of degenerating peripheral nerve cells, but also in the regulation of neurotransmitters and neuropeptides synthesis of sympathetic and sensory nerve cells. Exogenous NGF administration influences neuronal plasticity that allows the adult nervous system to modify its structure and functions in response to stimuli. Moreover, it was also demonstrated that the constitutive synthesis of NGF in adult tissues correlates with peripheral nervous system (PNS) neurons phenotypic features, such as innervation density, cell body size, axonal terminal sprouting, dendritic growth,

induction and/or inhibition of neuropeptides and neurotransmitters or transmitter-producing enzymes. NGF exerts its action on the growth and survival of peripheral sensory and sympathetic neurons and on a number of brain neurons, particularly basal forebrain cholinergic neurons (BFCN) that are among the major NGF-target cells within the central nervous system (CNS). The amino acid sequence of human NGF is available from GenBank: AAA59931.1, with amino acids 122 to 239 showing the mature peptide, which is also shown in the present disclosure as a part in SEQ ID NO. 2.

[0069] The term “PDGF” refers to platelet-derived growth factor, which is a potent mitogen and chemoattractant for many cells of mesenchymal origin, such as fibroblasts, activated macrophages, and smooth muscle cells. PDGF plays critical roles in angiogenesis, embryogenesis, inflammation, and cell differentiation; therefore, it can be beneficial in wound healing. The PDGF family of growth factors consists of four different polypeptide chains encoded by four different genes: the classical PDGF-A and PDGF-B chains, and the more recently discovered PDGF-C and PDGF-D. The four PDGF chains assemble into disulphide-bonded dimers via homo- or heterodimerization, and five different dimeric isoforms have been described so far: PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC and PDGF-DD. Two distinct PDGF receptors, alpha and beta, mediate the effects of the PDGFs on target cells. The PDGF-A and -C chains selectively bind the alpha receptor, whereas PDGF-D preferentially binds the beta receptor and PDGF-B displays a similar affinity for both receptors. Receptor activation requires PDGF-induced receptor dimerization, leading to transphosphorylation on tyrosine. PDGF AA induces only alpha/alpha receptor dimers, PDGF AB induces alpha/alpha and alpha/beta dimers, and PDGF BB induces all 3 combinations. Although PDGF-C binds only to the alpha receptor it can produce alpha-beta heterodimer formation by transactivation of beta receptor. A potential application of PDGF-BB protein is wound healing induction in diabetics. Furthermore, compositions comprising PDGF can facilitate the healing of injured or depleted bones by promoting the growth of the connective tissue, growth of the bone, and by stimulation of collagen synthesis in the injured bone region. The amino acid sequence of human PDGF-B is available from GenBank: CAA02294.1, with amino acids 82 to 190 showing the mature peptide, which is also shown in the present disclosure in SEQ ID NO. 3.

[0070] The term “innate defense regulatory peptide 1018 (IDR-1018)” is a cationic peptide with 12 amino acid residues (VRLIVAVRIWRR-NH₂). It has been investigated for its antimicrobial and immunomodulatory activities, because it has similarities with innate defense regulators. IDR-1018 has been demonstrated to have anti-infective, anti-inflammatory, wound healing (in rats), and anti-biofilm activities, reducing, for instance, the tumor necrosis factor alpha response induced by lipopolysaccharide.

- [0071] The term “improving wound healing” as used herein, unless otherwise specified, refers to obtaining an improved wound healing metric as compared with wound healing in the absence of the present methods and/or compositions. In certain embodiments, improved wound healing comprises improving fibroblast proliferation and/or improving fibroblast migration. In certain other embodiments, improved wound healing comprises increased wound closure and/or a reduction in the amount of time needed to complete wound healing. Those of ordinary skill in the art are familiar with other means of assessing improved wound healing and such means may be used to evidence improved wound healing according to the invention, for example through the use of scratch assay and/or a cell-proliferation assay, such as an epithelial proliferation assay.
- [0072] The tetraspanin protein family members, such as CD63, CD81, and CD9, ubiquitously expressed on exosomes and extensively used as exosome biomarkers, are involved in physiological processes, for instance cell adhesion, cell motility, and signal transduction. CD63, the first characterized tetraspanin, has two extracellular loops of unequal sizes and two short cytoplasmic domains, is involved in the signal transduction processes of various types of immune cells. Sequential domain deletion has identified the transmembrane helix 3 (TM3) being necessary and sufficient for membrane anchoring and exosome targeting. The amino acid sequence of human CD63 is available from e.g., GenBank: AHI51903.1, the amino acid sequence of TM3 of which corresponds to aa. 70 to 133, which is also shown as SEQ ID NO. 9 in the present disclosure.
- [0073] The term “anchoring polypeptide” is a polypeptide that is anchored on the exosome membrane when the exosome is generated by a cell. A transmembrane protein is a typical anchoring polypeptide in the context of the present disclosure. By “anchoring” or its grammatical variants, it means that at least a fragment of the polypeptide is embedded in the exosome membrane. The anchoring polypeptide may be fully or partly embedded in the exosome membrane. In this disclosure, the anchoring polypeptide is fused with a polypeptide heterologous to the exosome naturally produced by the same cell, such as the EGF polypeptide. Exemplary anchoring polypeptides are membrane proteins of exosome, membrane-targeting sequences, or an anchoring functional fragment thereof. Exemplary membrane proteins of exosome include but are not limited to lamp2b, tetraspanins such as CD63, CD9 and CD81, platelet-derived growth factor receptors (PDGFRs), lactadherin (C1C2 domain), vesicular stomatitis virus glycoprotein (VSVG), prostaglandin F2 receptor negative regulator (PTGFRN) and any combination thereof. Exemplary membrane-targeting sequences include but are not limited to glycosylphosphatidylinositol (GPI) anchors and lipid-anchored proteins. A detailed review regarding GPI anchors in exosome is

available from, e.g., Michel Vidal, Exosomes and GPI-anchored proteins: Judicious pairs for investigating biomarkers from body fluids, Advanced Drug Delivery Reviews, Volumes 161–162, 2020 (incorporated herein by reference in its entirety) . In a preferable embodiment of the present disclosure, the anchoring polypeptide comprises or consists of the transmembrane helix 3 (TM3) of CD63 protein.

[0074] Engineered Exosomes

[0075] One aspect of the present disclosure relates to an engineered exosome, comprising (a) an EGF polypeptide, fused to a first anchoring polypeptide, (b) a NGF polypeptide, fused to a second anchoring polypeptide, and (c) a PDGF-BB polypeptide, fused to a third anchoring polypeptide, wherein (a) , (b) and (c) are anchored on a membrane of the exosome via the first, second and third anchoring polypeptide, respectively, and wherein the EGF polypeptide, the NGF polypeptide and the PDGF-BB polypeptide are exposed on an outer surface of the membrane of the exosome.

[0076] In preferable embodiments, the engineered exosome provided by the present disclosure comprises (a) an EGF polypeptide, fused to a first anchoring polypeptide, (b) a NGF polypeptide, fused to a second anchoring polypeptide, (c) a PDGF-BB polypeptide, fused to a third anchoring polypeptide, and (d) an IDR-1018 polypeptide, fused to a fourth anchoring polypeptide, wherein (a) , (b) , (c) and (d) are anchored on a membrane of the exosome via the first, second, third and fourth anchoring polypeptide, respectively, and wherein the EGF polypeptide, the NGF polypeptide, the PDGF-BB polypeptide, and the IDR-1018 polypeptide are exposed on an outer surface of the membrane of the exosome.

[0077] In the present disclosure, the EGF polypeptide is a human EGF polypeptide or an ortholog or paralog thereof. In some embodiments, the EGF polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence as shown in SEQ ID NO. 1. In some embodiments, the EGF polypeptide comprises an amino acid sequence as shown in SEQ ID NO. 1. In some embodiments, the EGF polypeptide consists essentially of an amino acid sequence as shown in SEQ ID NO. 1.

[0078] In preferable embodiments, the NGF polypeptide is a human NGF polypeptide or an ortholog or paralog thereof. In some embodiments, the NGF polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence as shown in SEQ ID NO. 2. In some embodiments, the NGF polypeptide comprises an amino acid sequence as shown in SEQ ID NO. 2. In some embodiments, the NGF polypeptide consists essentially of an amino acid sequence as shown in SEQ ID NO. 2.

[0079] In preferable embodiments, the PDGF-BB polypeptide is a human PDGF-BB dimer or an ortholog or paralog thereof. In some embodiments, the PDGF-BB polypeptide

is a human PDGF-BB dimer. In some embodiments, the PDGF-BB polypeptide comprises an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence as shown in SEQ ID NO. 3. In some embodiments, the PDGF-BB polypeptide comprises an amino acid sequence as shown in SEQ ID NO. 3. In some embodiments, the PDGF-BB polypeptide consists essentially of an amino acid sequence as shown in SEQ ID NO. 3.

[0080] In some embodiments, the anchoring polypeptides are membrane proteins of exosome, membrane-targeting sequences, or an anchoring functional fragment thereof. Exemplary membrane proteins of exosome include but are not limited to lamp2b, tetraspanins such as CD63, CD9 and CD81, platelet-derived growth factor receptors (PDGFRs), lactadherin (C1C2 domain), vesicular stomatitis virus glycoprotein (VSVG), prostaglandin F2 receptor negative regulator (PTGFRN) and any combination thereof. Exemplary membrane-targeting sequences include but are not limited to glycosylphosphatidylinositol (GPI) anchors and lipid-anchored proteins. In preferable embodiments of the present disclosure, the first, second, third, and fourth anchoring polypeptides comprise a TM3 domain of CD63. In preferable embodiments, each of the first, second, third, and fourth anchoring polypeptides comprises a TM3 domain of CD63. In some embodiments, the anchoring polypeptides are full-length CD63 proteins, e.g., full-length human CD63 (see e.g., UniProtKB/Swiss-Prot: F8VZE2, P08962, Q5TZP3, Q8N6Z9, or Q9UCG6). In some embodiments, the anchoring polypeptides are truncated CD63 proteins that comprise a TM3 domain and at least one of TM1, TM2, and TM4 domains. For example, the anchoring polypeptide may consist of TM2 and TM3 of CD63; TM3 and TM4 of CD63; or TM1, TM2, and TM3 of CD63. In some embodiments, the anchoring polypeptides consist of TM3 domain of CD63. In the present disclosure, the first, second, third, and fourth anchoring polypeptides can be different or same. In preferable embodiments, the first, second, third, and fourth anchoring polypeptides are same and consist of TM3 domain of CD63.

[0081] In preferable embodiments, the TM3 domain of CD63 comprises an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence as shown in SEQ ID NO. 9. In preferable embodiments, the TM3 domain of CD63 comprises an amino acid sequence as shown in SEQ ID NO. 9. In preferable embodiments, the TM3 domain of CD63 consists essentially of an amino acid sequence as shown in SEQ ID NO. 9.

[0082] Therefore, in preferable embodiments, provided is an engineered exosome comprising (a) an EGF polypeptide, fused to a first anchoring polypeptide, (b) a NGF polypeptide, fused to a second anchoring polypeptide, and (c) PDGF-BB polypeptide,

fused to a third anchoring polypeptide, wherein each of the first, second, and third anchoring polypeptides comprises a TM3 domain of CD63, wherein (a), (b) and (c) are anchored on a membrane of the exosome via the first, second and third anchoring polypeptide, respectively, and wherein the EGF polypeptide, the NGF polypeptide and the PDGF-BB polypeptide are exposed on an outer surface of the membrane of the exosome.

[0083] In preferable embodiments, provided is an engineered exosome comprising (a) a human EGF polypeptide, fused to a first anchoring polypeptide, (b) a human NGF polypeptide, fused to a second anchoring polypeptide, and (c) a human PDGF-BB polypeptide, fused to a third anchoring polypeptide, wherein each of the first, second, and third anchoring polypeptides comprises a TM3 domain of CD63, wherein (a), (b) and (c) are anchored on a membrane of the exosome via the first, second and third anchoring polypeptide, respectively, and wherein the human EGF polypeptide, the human NGF polypeptide and the human PDGF-BB polypeptide are exposed on an outer surface of the membrane of the exosome.

[0084] In preferable embodiments, provided is an engineered exosome comprising (a) an EGF polypeptide comprising an amino acid sequence having at least 80% identity to the amino acid sequence as shown in SEQ ID NO. 1, fused to a first anchoring polypeptide, (b) a NGF polypeptide comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence as shown in SEQ ID NO. 2, fused to a second anchoring polypeptide, and (c) a PDGF-BB polypeptide comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence as shown in SEQ ID NO. 3, fused to a third anchoring polypeptide, wherein each of the first, second, and third anchoring polypeptides comprises a TM3 domain of CD63, wherein (a), (b) and (c) are anchored on a membrane of the exosome via the first, second and third anchoring polypeptide, respectively, and wherein the EGF polypeptide, the NGF polypeptide and the PDGF-BB polypeptide are exposed on an outer surface of the membrane of the exosome.

[0085] In preferable embodiments, provided is an engineered exosome comprising (a) an EGF polypeptide comprising an amino acid sequence having at least 80% identity to the amino acid sequence as shown in SEQ ID NO. 1, fused to a first anchoring polypeptide, (b) a NGF polypeptide comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence as shown in SEQ ID NO. 2, fused to a second anchoring polypeptide, and (c) a PDGF-BB polypeptide comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence as shown in SEQ ID NO. 3, fused to a

third anchoring polypeptide, wherein each of the first, second, and third anchoring polypeptides comprises a TM3 domain of CD63 comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence as shown in SEQ ID NO. 9, wherein (a), (b) and (c) are anchored on a membrane of the exosome via the first, second and third anchoring polypeptide, respectively, and wherein the EGF polypeptide, the NGF polypeptide and the PDGF-BB polypeptide are exposed on an outer surface of the membrane of the exosome.

[0086] In preferable embodiments, provided is an engineered exosome comprising (a) an EGF polypeptide comprising an amino acid sequence having an amino acid sequence as shown in SEQ ID NO. 1, fused to a first anchoring polypeptide, (b) a NGF polypeptide comprising an amino acid sequence having an amino acid sequence as shown in SEQ ID NO. 2, fused to a second anchoring polypeptide, and (c) a PDGF-BB polypeptide comprising an amino acid sequence having an amino acid sequence as shown in SEQ ID NO. 3, fused to a third anchoring polypeptide, wherein each of the first, second, and third anchoring polypeptides consists of a TM3 domain of CD63 having an amino acid sequence as shown in SEQ ID NO. 9, wherein (a), (b) and (c) are anchored on a membrane of the exosome via the first, second and third anchoring polypeptide, respectively, and wherein the EGF polypeptide, the NGF polypeptide and the PDGF-BB polypeptide are exposed on an outer surface of the membrane of the exosome.

[0087] In preferable embodiments, provided is an engineered exosome comprising (a) an EGF polypeptide, fused to a first anchoring polypeptide, (b) a NGF polypeptide, fused to a second anchoring polypeptide, (c) PDGF-BB polypeptide, fused to a third anchoring polypeptide, and (d) an IDR-1018 polypeptide, fused to a fourth anchoring polypeptide, wherein each of the first, second, third and fourth anchoring polypeptides comprises a TM3 domain of CD63, wherein (a), (b), (c) and (d) are anchored on a membrane of the exosome via the first, second, third and fourth anchoring polypeptide, respectively, and wherein the EGF polypeptide, the NGF polypeptide, the PDGF-BB polypeptide and the IDR-1018 polypeptide are exposed on an outer surface of the membrane of the exosome.

[0088] In preferable embodiments, provided is an engineered exosome comprising (a) a human EGF polypeptide, fused to a first anchoring polypeptide, (b) a human NGF polypeptide, fused to a second anchoring polypeptide, (c) a human PDGF-BB polypeptide, fused to a third anchoring polypeptide, and (d) a human IDR-1018 polypeptide, fused to a fourth anchoring polypeptide, wherein each of the first, second, third and fourth anchoring polypeptides comprises a TM3 domain of CD63, wherein (a), (b), (c) and (d) are anchored on a membrane of the exosome via the

first, second, third and fourth anchoring polypeptide, respectively, and wherein the human EGF polypeptide, the human NGF polypeptide, the PDGF-BB polypeptide and the IDR-1018 polypeptide are exposed on an outer surface of the membrane of the exosome.

[0089] In preferable embodiments, provided is an engineered exosome comprising (a) an EGF polypeptide comprising an amino acid sequence having at least 80% identity to the amino acid sequence as shown in SEQ ID NO. 1, fused to a first anchoring polypeptide, (b) a NGF polypeptide comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence as shown in SEQ ID NO. 2, fused to a second anchoring polypeptide, (c) a PDGF-BB polypeptide comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence as shown in SEQ ID NO. 3, fused to a third anchoring polypeptide, and (d) an IDR-1018 polypeptide comprising an amino acid sequence as shown in SEQ ID NO. 4, fused to a fourth anchoring polypeptide, wherein each of the first, second, third and fourth anchoring polypeptides comprises a TM3 domain of CD63, wherein (a), (b), (c) and (d) are anchored on a membrane of the exosome via the first, second, third and fourth anchoring polypeptide, respectively, and wherein the EGF polypeptide, the NGF polypeptide, the PDGF-BB polypeptide, and the IDR-1018 polypeptide are exposed on an outer surface of the membrane of the exosome.

[0090] In preferable embodiments, provided is an engineered exosome comprising (a) an EGF polypeptide comprising an amino acid sequence having at least 80% identity to the amino acid sequence as shown in SEQ ID NO. 1, fused to a first anchoring polypeptide, (b) a NGF polypeptide comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence as shown in SEQ ID NO. 2, fused to a second anchoring polypeptide, (c) a PDGF-BB polypeptide comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence as shown in SEQ ID NO. 3, fused to a third anchoring polypeptide, and (d) an IDR-1018 polypeptide comprising an amino acid sequence as shown in SEQ ID NO. 4, fused to a fourth anchoring polypeptide, wherein each of the first, second, third and fourth anchoring polypeptides comprises a TM3 domain of CD63 comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to the amino acid sequence as shown in SEQ ID NO. 9, wherein (a), (b), (c) and (d) are anchored on a membrane of the exosome via the first, second, third and fourth anchoring polypeptide, respectively, and wherein the EGF polypeptide, the NGF polypeptide, the PDGF-BB

polypeptide, and the IDR-1018 polypeptide are exposed on an outer surface of the membrane of the exosome.

- [0091] In preferable embodiments, provided is an engineered exosome comprising (a) an EGF polypeptide comprising an amino acid sequence having an amino acid sequence as shown in SEQ ID NO. 1, fused to a first anchoring polypeptide, (b) a NGF polypeptide comprising an amino acid sequence having an amino acid sequence as shown in SEQ ID NO. 2, fused to a second anchoring polypeptide, (c) a PDGF-BB polypeptide comprising an amino acid sequence having an amino acid sequence as shown in SEQ ID NO. 3, fused to a third anchoring polypeptide, and (d) an IDR-1018 polypeptide comprising an amino acid sequence as shown in SEQ ID NO. 4, wherein each of the first, second, third and fourth anchoring polypeptides consists of a TM3 domain of CD63 having an amino acid sequence as shown in SEQ ID NO. 9, wherein (a), (b), (c) and (d) are anchored on a membrane of the exosome via the first, second, third and fourth anchoring polypeptide, respectively, and wherein the EGF polypeptide, the NGF polypeptide, the PDGF-BB polypeptide, and the IDR-1018 polypeptide are exposed on an outer surface of the membrane of the exosome.
- [0092] In any of the embodiments described above, the EGF polypeptide, the NGF polypeptide, the PDGF-BB polypeptide, and, when present, the IDR-1018 polypeptide, is fused to the C-terminus of the first, second, third, and fourth anchoring polypeptides, respectively, directly or through a peptide linker. The peptide linker could be any peptide linker that is available in the art useful for linking different domains or functional regions in a fusion protein. In preferable embodiments, the peptide linker consists of glycine and serine, e.g., (G4S)_n, in which n is an integer from 1 to 3.
- [0093] In the present disclosure, the exosome is preferably not derived from a mesenchymal stem cell. More preferably, the exosome is not derived from a stem cell. In preferable embodiments, the exosome provided by the present invention is derived from a non-stem cell, such as a CHO or HEK293 cell. In the present disclosure, the exosome is preferably purified and/or isolated from the cell from which it is derived.
- [0094] The engineered exosomes provided by the present invention have shown significant wound healing improvement, with some exosome treatment comparable to healthy control. These results were surprising because chronic wound, such as diabetic foot ulcer is extremely intractable with altered wound healing kinetics compared to acute wound. It is contemplated that these results were achieved at least partly through the combination of the polypeptides presented onto the exosome. In the present disclosure, at least three polypeptides (i.e., EGF, NGF, and PDGF-BB) are presented onto the surface of the exosomes through the anchoring polypeptides. It is contemplated that the combination of the polypeptides is superior to any single polypeptide of them when displayed onto the surface of the exosome. For example, an engineered exosome

displaying EGF, NGF and PDGF-BB is significantly more effective than an engineered exosome displaying EGF, NGF, or PDGF-BB alone, in terms of treating wounds or improving wound healing.

[0095] Methods and Compositions for Improving Wound Healing

[0096] Aspects of the present disclosure are directed to methods and compositions for improving wound healing or for treating a wound. In some embodiments, the wound is an acute wound, such as deep burns, massive skin loss, surgical interventions, or radiotherapy-induced injuries. In preferable embodiments, the wound is a chronic wound. In preferable embodiment, the chronic wound is a non-healing ulcer or a diabetic wound; more preferably, the wound includes diabetic foot ulcer.

[0097] In preferable embodiments, provided are method of treating a chronic wound comprising administering to a subject in need thereof a therapeutically effective amount of the engineered exosome or the composition disclosed herein.

[0098] In preferable embodiments, provided are method of treating a diabetic foot ulcer comprising administering to a subject in need thereof a therapeutically effective amount of the engineered exosome or the composition disclosed herein.

[0099] In the present disclosure, the composition provided comprises the engineered exosome and a carrier. In preferable embodiments, the composition is a liquid formulation. In preferable embodiment, the composition is formulated for topical or intradermal administration. In preferable embodiment, the composition is a pharmaceutical composition comprising a pharmaceutically acceptable carrier. In preferable embodiment, the composition is a cosmetic composition comprising a cosmetically acceptable carrier.

[0100] In preferable embodiments, the composition does not contain an EGF polypeptide, a NGF polypeptide, a PDGF-BB polypeptide, or IDR-1018 polypeptide, not attached to the membrane of the exosome (i.e., free polypeptides) . For example, no additional EGF polypeptide, a NGF polypeptide, a PDGF-BB polypeptide, or IDR-1018 polypeptide is added or supplemented to the composition except for the polypeptides anchored to the membrane of the engineered exosomes.

[0101] In some embodiments, the composition is a cosmetic composition. In some embodiments, the composition is a non-cosmetic composition. In some embodiments, the composition is a pharmaceutical composition.

[0102] Nucleic Acid Constructs, Vectors, Cells, and Methods of Production

[0103] Aspects of the present disclosure relate also to nucleic acid constructs that encode the polypeptides anchored on the exosomes.

[0104] In some embodiments, provided is a nucleic acid construct comprising a polynucleotide encoding (a) an EGF polypeptide fused to a first anchoring polypeptide, (b)

a NGF polypeptide fused to a second anchoring polypeptide, and (c) a PDGF-BB polypeptide, fused to a third anchoring polypeptide.

- [0105] In some embodiments, provided is a nucleic acid construct comprising a polynucleotide encoding (a) an EGF polypeptide fused to a first anchoring polypeptide, (b) a NGF polypeptide fused to a second anchoring polypeptide, (c) a PDGF-BB polypeptide, fused to a third anchoring polypeptide, and (d) an IDR-1018 polypeptide, fused to a fourth anchoring polypeptide.
- [0106] In some embodiments, provided are a set of three nucleic acid constructs, with a first nucleic acid construct comprising a polynucleotide encoding (a) , a second nucleic acid construct comprising a polynucleotide encoding (b) , and a third nucleic acid construct comprising a polynucleotide encoding (c) , wherein (a) , (b) , and (c) are defined as above.
- [0107] In some embodiments, provided are a set of four nucleic acid constructs, with a first nucleic acid construct comprising a polynucleotide encoding (a) , a second nucleic acid construct comprising a polynucleotide encoding (b) , a third nucleic acid construct comprising a polynucleotide encoding (c) , and a fourth nucleic acid construct comprising a polynucleotide encoding (d) , wherein (a) , (b) , (c) , and (d) are defined as above.
- [0108] In some embodiments, provided are a set of two nucleic acid constructs, with one nucleic acid construct comprising a polynucleotide encoding two of (a) , (b) and (c) , and the other nucleic acid construct comprising a polynucleotide encoding the remaining, wherein (a) , (b) , and (c) are defined as above.
- [0109] In some embodiments, provided are a set of two nucleic acid constructs, with one nucleic acid construct comprising a polynucleotide encoding two of (a) , (b) , (c) and (d) , and the other nucleic acid construct comprising a polynucleotide encoding the other two, wherein (a) , (b) , (c) and (d) are defined as above.
- [0110] In some embodiments, provided are a set of three nucleic acid constructs, with a first nucleic acid construct comprising a polynucleotide encoding two of (a) , (b) , (c) and (d) , a second nucleic acid construct comprising a polynucleotide encoding one of the remaining polypeptide, and a third nucleic acid construct comprising a polynucleotide encoding the other of the remaining polypeptide, wherein (a) , (b) , (c) and (d) are defined as above.
- [0111] In preferable embodiments, provided is a single nucleic acid construct comprising a polynucleotide encoding (a) , (b) , and (c) , wherein (a) , (b) , and (c) are defined as above.
- [0112] In preferable embodiments, provided is a single nucleic acid construct comprising a polynucleotide encoding (a) , (b) , (c) and (d) , wherein (a) , (b) , (c) and (d) are defined as above.

- [0113] In some embodiments, the anchoring polypeptides are membrane proteins of exosome, membrane-targeting sequences, or an anchoring functionally fragment thereof. Exemplary membrane proteins of exosome include but are not limited to lamp2b, tetraspanins such as CD63, CD9 and CD81, platelet-derived growth factor receptors (PDGFRs), lactadherin (C1C2 domain), vesicular stomatitis virus glycoprotein (VSVG), prostaglandin F2 receptor negative regulator (PTGFRN) and any combination thereof. Exemplary membrane-targeting sequences include but are not limited to glycosylphosphatidylinositol (GPI) anchors and lipid-anchored proteins.
- [0114] In some embodiments, each of the anchoring polypeptide may be located at the N terminus of the polypeptides to be presented on the surface of the exosome (e.g., NGF, EGF, PDGF-BB and IDR-1018), to ensure that the latter are exposed on the surface of the exosome. In some embodiments, each of the anchoring polypeptide may be located at the C terminus of the polypeptides to be presented on the surface of the exosome, to ensure that the latter are exposed on the surface of the exosome. For example, when lamp2b is used as one of the anchoring polypeptides, the polypeptides (e.g., NGF, EGF, PDGF-BB or IDR-1018) to be presented on the surface of the exosome may be located at N terminus of lamp2b. For example, when TM3 domain of CD63 is used as one of the anchoring polypeptides, the polypeptides (e.g., NGF, EGF, PDGF-BB or IDR-1018) to be presented on the surface of the exosome may be located at C terminus of the TM3 domain of CD63. In some embodiments, when different anchoring polypeptides are used, the polypeptides to be presented on the surface of the exosome may be located at either N or C terminus of the anchoring polypeptides, dependent on the species of the anchoring polypeptides used.
- [0115] In preferable embodiments, the anchoring polypeptides comprise a full-length CD63 or a truncated CD63 that retains TM3 domain. In preferable embodiments, each of the anchoring polypeptides comprises a TM3 domain of CD63. In preferable embodiments, each of the first, second, third, and fourth anchoring polypeptides is a TM3 domain of CD63.
- [0116] In the preferable embodiments, the polynucleotide may comprise, from 5' to 3', a fragment of nucleotides encoding:
- [0117] (i) [1st anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [PDGF-BB],
- [0118] (ii) [1st anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [NGF],

- [0119] (iii) [1st anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [PDGF-BB] ,
- [0120] (iv) [1st anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [EGF] ,
- [0121] (v) [1st anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [NGF] , or
- [0122] (vi) [1st anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [EGF] ,
- [0123] wherein, [] represents a separate polypeptide, and] - [represents a linker or a bond.
- [0124] In preferable embodiments, the self-cleavage peptide is a 2A peptide, e.g., T2A, E2A, P2A or any combination thereof. For example, the self-cleavage peptide is a T2A peptide. The self-cleavage peptide is cleaved after the polynucleotide is translated, resulting in three or four independent fusion proteins, each comprising a single polypeptide to be presented onto the surface of the exosome and a single anchoring polypeptide.
- [0125] In preferable embodiments, provided is a single nucleic acid construct comprising a polynucleotide encoding (a) , (b) , and (c) , wherein (a) , (b) , and (c) are defined as above, and wherein each of the first, second, and third anchoring polypeptides comprises a TM3 domain of CD63, the polynucleotide may comprise, from 5' to 3' , a fragment of nucleotides encoding one of
- [0126] (i) [TM3] - [EGF] - [T2A] - [TM3] - [NGF] - [T2A] - [TM3] - [PDGF-BB] ,
- [0127] (ii) [TM3] - [EGF] - [T2A] - [TM3] - [PDGF-BB] - [T2A] - [TM3] - [NGF] ,
- [0128] (iii) [TM3] - [NGF] - [T2A] - [TM3] - [EGF] - [T2A] - [TM3] - [PDGF-BB] ,
- [0129] (iv) [TM3] - [NGF] - [T2A] - [TM3] - [PDGF-BB] - [T2A] - [TM3] - [EGF] ,
- [0130] (v) [TM3] - [PDGF-BB] - [T2A] - [TM3] - [EGF] - [T2A] - [TM3] - [NGF] , and
- [0131] (vi) [TM3] - [PDGF-BB] - [T2A] - [TM3] - [NGF] - [T2A] - [TM3] - [EGF] ,
- [0132] wherein, [] represents a separate polypeptide, and] - [represents a linker or a bond; and wherein TM3 represents TM3 domain of CD63; and T2A represents self-cleavage peptide T2A.
- [0133] In preferable embodiments, the polynucleotide may comprise, from 5' to 3' , a fragment of nucleotides encoding:

- [0134] (a) [1st anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [4th anchoring polypeptide] - [IDR-1018] ,
- [0135] (b) [1st anchoring polypeptide] - [IDR-1018] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [4th anchoring polypeptide] - [PDGF-BB] ,
- [0136] (c) [1st anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [IDR-1018] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [4th anchoring polypeptide] - [PDGF-BB] ,
- [0137] (d) [1st anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [IDR-1018] - [self-cleavage peptide] - [4th anchoring polypeptide] - [PDGF-BB] ,
- [0138] (e) [1st anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [4th anchoring polypeptide] - [IDR-1018] ,
- [0139] (f) [1st anchoring polypeptide] - [IDR-1018] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [4th anchoring polypeptide] - [NGF] ,
- [0140] (g) [1st anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [IDR-1018] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [4th anchoring polypeptide] - [NGF] ,
- [0141] (h) [1st anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [IDR-1018] - [self-cleavage peptide] - [4th anchoring polypeptide] - [NGF] ,
- [0142] (i) [1st anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [4th anchoring polypeptide] - [IDR-1018] ,
- [0143] (j) [1st anchoring polypeptide] - [IDR-1018] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [4th anchoring polypeptide] - [PDGF-BB] ,
- [0144] (k) [1st anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [IDR-1018] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [4th anchoring polypeptide] - [PDGF-BB] ,

- [0145] (l) [1st anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [IDR-1018] - [self-cleavage peptide] - [4th anchoring polypeptide] - [PDGF-BB] ,
- [0146] (m) [1st anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [4th anchoring polypeptide] - [IDR-1018] ,
- [0147] (n) [1st anchoring polypeptide] - [IDR-1018] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [4th anchoring polypeptide] - [EGF] ,
- [0148] (o) [1st anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [IDR-1018] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [4th anchoring polypeptide] - [EGF] ,
- [0149] (p) [1st anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [IDR-1018] - [self-cleavage peptide] - [4th anchoring polypeptide] - [EGF] ,
- [0150] (q) [1st anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [4th anchoring polypeptide] - [IDR-1018] ,
- [0151] (r) [1st anchoring polypeptide] - [IDR-1018] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [EGF] [self-cleavage peptide] - [4th anchoring polypeptide] - [NGF] ,
- [0152] (s) [1st anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [IDR-1018] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [EGF] [self-cleavage peptide] - [4th anchoring polypeptide] - [NGF] ,
- [0153] (t) [1st anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [IDR-1018] [self-cleavage peptide] - [4th anchoring polypeptide] - [NGF] ,
- [0154] (u) [1st anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [EGF] - [self-cleavage peptide] - [4th anchoring polypeptide] - [IDR-1018] ,
- [0155] (v) [1st anchoring polypeptide] - [IDR-1018] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [4th anchoring polypeptide] - [EGF] ,
- [0156] (w) [1st anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [IDR-1018] - [self-cleavage peptide] - [3rd anchoring

polypeptide] - [NGF] - [self-cleavage peptide] - [4th anchoring polypeptide] - [EGF] ,
or

- [0157] (x) [1st anchoring polypeptide] - [PDGF-BB] - [self-cleavage peptide] - [2nd anchoring polypeptide] - [NGF] - [self-cleavage peptide] - [3rd anchoring polypeptide] - [IDR-1018] - [self-cleavage peptide] - [4th anchoring polypeptide] - [EGF] ,
- [0158] wherein, [] represents a separate polypeptide, and] - [represents a linker or a bond.
- [0159] In preferable embodiments, the self-cleavage peptide is a 2A peptide, e.g., T2A, E2A, P2A or any combination thereof. For example, the self-cleavage peptide is a T2A peptide.
- [0160] In other embodiments, the single polynucleotide encodes at least an anchoring polypeptide that is located at the C terminus of the polypeptide to be presented onto the surface of the exosome. In some embodiments, each of the first, second, third, and fourth anchoring polypeptides are different and thus the anchoring polypeptides may locate at N or C terminus of the polypeptides EGF, NGF, PDGF-BB, and, optionally, IDR-1018.
- [0161] In preferable embodiments, provided is a single nucleic acid construct comprising a polynucleotide encoding (a) , (b) , (c) and (d) , wherein (a) , (b) , (c) and (d) are defined as above, and wherein each of the first, second, third, and fourth anchoring polypeptides comprises a TM3 domain of CD63, the polynucleotide may comprise, from 5' to 3' , a fragment of nucleotides encoding one of
- [0162] [TM3] - [EGF] - [T2A] - [TM3] - [NGF] - [T2A] - [TM3] - [PDGF-BB] - [T2A] - [TM3] - [IDR-1018] ,
- [0163] [TM3] - [IDR-1018] - [T2A] - [TM3] - [EGF] - [T2A] - [TM3] - [NGF] - [T2A] - [TM3] - [PDGF-BB] ,
- [0164] [TM3] - [EGF] - [T2A] - [TM3] - [IDR-1018] - [T2A] - [TM3] - [NGF] - [T2A] - [TM3] - [PDGF-BB] ,
- [0165] [TM3] - [EGF] - [T2A] - [TM3] - [NGF] - [T2A] - [TM3] - [IDR-1018] - [T2A] - [TM3] - [PDGF-BB] ,
- [0166] [TM3] - [EGF] - [T2A] - [TM3] - [PDGF-BB] - [T2A] - [TM3] - [NGF] - [T2A] - [TM3] - [IDR-1018] ,
- [0167] [TM3] - [IDR-1018] - [T2A] - [TM3] - [EGF] - [T2A] - [TM3] - [PDGF-BB] - [T2A] - [TM3] - [NGF] ,
- [0168] [TM3] - [EGF] - [T2A] - [TM3] - [IDR-1018] - [T2A] - [TM3] - [PDGF-BB] - [T2A] - [TM3] - [NGF] ,
- [0169] [TM3] - [EGF] - [T2A] - [TM3] - [PDGF-BB] - [T2A] - [TM3] - [IDR-1018] - [T2A] - [TM3] - [NGF] ,

- [0170] [TM3] - [NGF] - [T2A] - [TM3] - [EGF] - [T2A] - [TM3] - [PDGF-BB] - [T2A] - [TM3] - [IDR-1018] ,
- [0171] [TM3] - [IDR-1018] - [T2A] - [TM3] - [NGF] - [T2A] - [TM3] - [EGF] - [T2A] - [TM3] - [PDGF-BB] ,
- [0172] [TM3] - [NGF] - [T2A] - [TM3] - [IDR-1018] - [T2A] - [TM3] - [EGF] - [T2A] - [TM3] - [PDGF-BB] ,
- [0173] [TM3] - [NGF] - [T2A] - [TM3] - [EGF] - [T2A] - [TM3] - [IDR-1018] - [T2A] - [TM3] - [PDGF-BB] ,
- [0174] [TM3] - [NGF] - [T2A] - [TM3] - [PDGF-BB] - [T2A] - [TM3] - [EGF] - [T2A] - [TM3] - [IDR-1018] ,
- [0175] [TM3] - [IDR-1018] - [T2A] - [TM3] - [NGF] - [T2A] - [TM3] - [PDGF-BB] - [T2A] - [TM3] - [EGF] ,
- [0176] [TM3] - [NGF] - [T2A] - [TM3] - [IDR-1018] - [T2A] - [TM3] - [PDGF-BB] - [T2A] - [TM3] - [EGF] ,
- [0177] [TM3] - [NGF] - [T2A] - [TM3] - [PDGF-BB] - [T2A] - [TM3] - [IDR-1018] - [T2A] - [TM3] - [EGF] ,
- [0178] [TM3] - [PDGF-BB] - [T2A] - [TM3] - [EGF] - [T2A] - [TM3] - [NGF] - [T2A] - [TM3] - [IDR-1018] ,
- [0179] [TM3] - [IDR-1018] - [T2A] - [TM3] - [PDGF-BB] - [T2A] - [TM3] - [EGF] - [T2A] - [TM3] - [NGF] ,
- [0180] [TM3] - [PDGF-BB] - [T2A] - [TM3] - [IDR-1018] - [T2A] - [TM3] - [EGF] - [T2A] - [TM3] - [NGF] ,
- [0181] [TM3] - [PDGF-BB] - [T2A] - [TM3] - [EGF] - [T2A] - [TM3] - [IDR-1018] - [T2A] - [TM3] - [NGF] ,
- [0182] [TM3] - [PDGF-BB] - [T2A] - [TM3] - [NGF] - [T2A] - [TM3] - [EGF] - [T2A] - [TM3] - [IDR-1018] ,
- [0183] [TM3] - [IDR-1018] - [T2A] - [TM3] - [PDGF-BB] - [T2A] - [TM3] - [NGF] - [T2A] - [TM3] - [EGF] ,
- [0184] [TM3] - [PDGF-BB] - [T2A] - [TM3] - [IDR-1018] - [T2A] - [TM3] - [NGF] - [T2A] - [TM3] - [EGF] , or
- [0185] [TM3] - [PDGF-BB] - [T2A] - [TM3] - [NGF] - [T2A] - [TM3] - [IDR-1018] - [T2A] - [TM3] - [EGF]
- [0186] wherein, [] represents a separate polypeptide, and] - [represents a linker or a bond; and wherein TM3 represents TM3 domain of CD63; and T2A represents self-cleavage peptide T2A.
- [0187] In this section, the EGF, NGF, PDGF-BB, and IDR-1018 polypeptide have the meaning and preferable embodiments given above in reference to the section titled Engineered Exosomes.

- [0188] In some embodiments, a polynucleotide encoding the EGF polypeptide has a nucleotide sequence as shown in SEQ ID NO. 5 or a degenerate sequence thereof.
- [0189] In some embodiments, a polynucleotide encoding the NGF polypeptide has a nucleotide sequence as shown in SEQ ID NO. 6 or a degenerate sequence thereof.
- [0190] In some embodiments, a polynucleotide encoding the PDGF-BB polypeptide has a nucleotide sequence as shown in SEQ ID NO. 7 or a degenerate sequence thereof.
- [0191] In some embodiments, a polynucleotide encoding the IDR-1018 polypeptide has a nucleotide sequence as shown in SEQ ID NO. 8 or a degenerate sequence thereof.
- [0192] In some embodiments, a polynucleotide encoding the TM3 domain of CD63 has a nucleotide sequence as shown in SEQ ID NO. 10 or a degenerate sequence thereof.
- [0193] Also provided are vectors that comprise the nucleic acid construct (s) described above. In some embodiments, the vector is a viral vector. In preferable embodiments, the vector is a lentiviral vector or an adeno-associated viral vector.
- [0194] The vectors provided herein facilitate integration of the polynucleotides encoding the polypeptides anchored on the membrane of the engineered exosomes into the genome of the cells producing the exosomes.
- [0195] Also provided are cells transduced with the vectors. In preferable embodiments, the cell is not a mesenchymal stem cell. In preferable embodiments, the cell is not a stem cell. In preferable embodiments, the cell is a non-stem cell, such as a HEK293 or CHO cell.
- [0196] The present disclosure also provides a process of producing the engineered exosome provided herein, comprising transducing the cell described above, such as HEK293 cell, with the vector described above; culturing the cell in a condition allowing secretion of the engineered exosome from the cell; and collecting and purifying the engineered exosome.
- [0197] In some embodiments, the process comprises an adaption of the cell from serum-containing condition to a serum-free condition during culturing. The adaption may comprise a sequential adaptation with decreasing full medium and increasing serum-free medium.
- [0198] Sequences Listings

SEQ ID NO.	Description	Sequences
1	Human EGF AA	NSDSECLSHDGYCLHDGVCMYIEALDKYACNCVVGYI GERCQYRDLKWWELR
2	Human NGF AA	SSSHPIFHRGEFSVCDSVSVWVGDKTTATDIKGKEVMVL GEVNINNSVFKQYFFETKCRDPNPVDSGCRGIDSKHWNS YCTTTHTFVKALTMDGKQAAWRFIRIDTACVCVLSRKA VR
3	Human PDGF- BB AA	SLGSLTIAEPAMIAECKTRTEVFEISRRLIDRTNANFLVWP PCVEVQRCSGCCNNRNVQCRPTQVQLRPVQVRKIEIVRK KPIFKKATVTLEDHLACKCETVAAARPVT
4	IDR-1018 AA	VRLIVAVRIWRR
5	Human EGF DNA	AATTCCGATTCCGAGTGCCCTCTGTCCCACGATGGCTA CTGTCTGCACGATGGCGTGTGCATGTACATCGAGGCCC TGGACAAGTACGCCTGCAACTGCGTGGTGGGCTACAT CGGCGAGAGGTGCCAGTACAGGGACCTGAAGTGGTG GGAGCTGAGG
6	Human NGF DNA	AGCAGCTCCCACCCTATCTTTACAGGGGCGAGTTCAG CGTGTGTGACAGCGTGAGCGTGTGGGTGGGCGATAAG ACCACCGCCACCGATATCAAGGGCAAGGAGGTGATGG TGCTGGGCGAGGTGAACATCAACAACAGCGTGTTCAA GCAGTACTTTTTTGAGACCAAGTGCAGGGACCCAAAC CCTGTGGACTCCGGCTGCAGGGGCATCGATAGCAAGC

		ACTGGAATAGCTACTGTACCACAACCCACACATTTGTG AAGGCCCTGACCATGGACGGCAAGCAGGCCGCTGGA GGTTCATCAGGATCGACACCGCCTGTGTGTGTGTGCTG TCCAGGAAGGCCGTGAGG
7	Human PDGF- BB DNA	AGCCTGGGATCCCTGACAATCGCCGAGCCCGCCATGAT CGCCGAGTGCAAGACAAGGACAGAGGTGTTTGTAGATC TCCAGAAGACTGATCGACAGAACAATGCCAATTCCT GGTGTGGCCTCCTTGCCTGGAGGTGCAGAGGTGCTCC GGCTGCTGTAACAATAGAAACGTGCAGTGCAGACCCA CCCAGGTGCAGCTGAGGCCCGTGCAGGTGAGAAAGAT CGAGATCGTGAGGAAGAAGCCTATCTTTAAGAAGGCC ACCGTGACCCTGGAGGACCACCTGGCCTGCAAGTGCG AGACAGTGGCCGCCGCTAGACCCGTGACC
8	IDR-1018 DNA	GTGAGGCTGATCGTGGCCGTGAGAATCTGGAGAAGG
9	CD63 TM3 AA	YCLMITFAIFLSLIMLVEVAAAIAGYVFRDKVMSEFNNTF RQQMENYPKNNHTA
10	CD63 TM3 DNA	TACTGTCTGATGATCACCTTCGCCATCTTTCTGTCCCTG ATCATGCTGGTGGAGGTGGCCGCCGCCATCGCCGGATA CGTGTTTACAGAGACAAGGTGATGAGCGAGTTCAACAAT AATTTTAGACAGCAGATGGAGAATTACCCTAAGAATAA CCACACCGCC
11	Linker AA	GGGSGGGSGGGGS
12	Linker DNA	GGCGGCGGCGGCTCCGGAGGAGGAGGAAGCGGAGGA GGCGGCAGC
13	T2A AA	GSGEGRGSLTCDVEENPGP
14	T2A DNA	GGCAGCGGCGAAGGCCGCGGCAGCCTGCTGACCTGCG GCGATGTGGAAGAAAACCCGGGCCCCG
15	Rat EGF AA	NSNTGCPPSYDGYCLNGGVCMYVESVDRYVCNCVIGYI GERCQHRDLRWKLR
16	Rat NGF AA	SSTHPVFHMGFEFSVCDVSVVWVGDKTTATDIKGKEVT LGEVNNNSVFKQYFFETKCRAPNPVESGCRGIDSKHWN SYCTTTHTFVKALTTDDKQAAWRFIRIDTACVCVLSRKA AR
17	Rat PDGF-BB AA	SLGSLAAEPAVIAECKTRTEVFQISRNLIDRTNANFLVW PPCVEVQRCSGCCNNRNVQCRASQVQMRPVQVRKIEIV RKKPVFKKATVTLEDHLACKCETVVTTPRPVT
18	Rat EGF DNA	AACAGCAACACAGGCTGTCTCCTTCCTACGATGGCTA CTGCCTGAATGGCGGCGTGTGTATGTACGTGGAGTCCG TGGACAGATACGTGTGCAATTGCGTGATCGGCTACATC GGCGAGAGATGCCAGCACAGGGATCTGAGATGGTGGA AGCTGAGG
19	Rat NGF DNA	TCCTCCACCCACCCAGTGTTCACATGGGCGAGTTTAG CGTGTGTGACAGCGTGAGCGTGTGGGTGGGCGACAAG ACAACCGCCACAGACATCAAGGCAAGGAGGTGACC

		GTGCTGGGCGAGGTGAATATCAACAACCTCCGTGTTCA AGCAGTACTTCTTTGAGACAAAGTGTAGAGCTCCTAAC CCAGTGGAGTCCGGCTGCAGGGGCATCGACAGCAAGC ACTGGAATAGCTACTGCACAACAACCCACACCTTTGTG AAGGCCCTGACCACCGATGACAAGCAGGCCCGCTGGA GATTCATCAGAATCGATACCGCCTGTGTGTGTGTGCTG AGCAGGAAGGCCGCCAGA
20	Rat PDGF-BB DNA	AGCCTGGGATCCCTGGCTGCTGCCGAGCCCCGTGTGAT CGCCGAGTGCAAGACAAGGACAGAGGTGTTCCAGATC AGCAGGAACCTGATCGATAGAACCAACGCCAATTTTCT GGTGTGGCCTCCTTGTGTGGAGGTGCAGAGATGCAGC GGCTGTTGTAATAATAGGAATGTGCAGTGCAGGGCCAG CCAGGTGCAGATGAGACCTGTGCAGGTGAGAAAGATC GAGATCGTGAGGAAGAAGCCCGTGTTTAAGAAGGCCA CAGTGACACTGGAGGATCACCTGGCCTGTAAGTGCGA GACCGTGGTGACACCCAGGCCCGTGACA

AA: Amino Acid Sequence

Examples

- [0199] Example 1. Construction of engineered exosomes derived stable cell lines.
- [0200] Materials and Methods
- [0201] Materials: The HEK293 cell line (human embryonic kidney 293 cells, CRL-1573™) was purchased from ATCC, which were maintained in DMEM (high-glucose) containing 10% (vol/vol) FBS, supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin. The CHO-K1 cell line (Chinese Hamster Ovary Cell) was purchased from BeNa Culture Collection (Beijing, China) . CHO-K1 cells were maintained in F-12K (31765035, Thermo Fisher Scientific, United States) containing 10% (vol/vol) FBS, supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin. Cells were incubated in a humidified atmosphere containing 5%CO₂ at 37 °C. Antibody used in this study was anti-CD63 antibodies (Cat. No. MA5-32085, Invitrogen) .
- [0202] pGOI Plasmids Construction: The amino acid sequences of all the target genes, including EGF, NGF, PDGF-BB from both human and rat, as well as IDR-1018 were derived from Uniprot and the corresponding DNA sequences (see SEQ ID NO. 18-20, and 8, respectively, for rat genes) were synthesized by General Biotechnology (Chuzhou, China) with plasmid pCDH-CMV-MCS-EF1a-GFP+BSD (System Biosciences) . The T2A peptide (see SEQ ID NO. 13 and 14) was used for dissociating all the target proteins into individual protein upon translation. The 4 plasmids (i.e., pGOI, pGag/Pol, pRev, and pVSV-G) of 3rd-generation system were used for lentivirus production, with pCDH-CMV-MCS-EF1a-GFP+BSD (blasticidin resistance) as

lentivirus packaging plasmid. The lentiviruses were packaged by WZ Biotechnology (Jinan, China) .

- [0203] Payload genes constructed in two pGOI plasmids:
- [0204] No. 1: CD63-TM3-Linker-EGF, in which CD63-TM3 is the TM3 domain of CD63, encoded by DNA sequence shown in SEQ ID NO. 10, and EGF is encoded by a human EGF DNA as shown in SEQ ID NO. 5, respectively.
- [0205] No. 2: CD63-TM3-Linker-EGF, in which CD63-TM3 is the TM3 domain of CD63, encoded by DNA sequence shown in SEQ ID NO. 10, and EGF is encoded by a rat EGF DNA as shown in SEQ ID NO. 18, respectively.
- [0206] No. 37: CD63-TM3-Linker-EGF-T2A-CD63-TM3-Linker-NGF-T2A-CD63-TM3-Linker-PDGF-BB, in which each CD63-TM3 is the TM3 domain of CD63, encoded by DNA sequence shown in SEQ ID NO. 10, each linker is encoded by DNA sequence shown in SEQ ID NO. 12, and each T2A is encoded by DNA sequence shown in SEQ ID NO. 14; EGF, NGF, and PDGF-BB are encoded by a human EGF DNA as shown in SEQ ID NO. 5, a human NGF DNA as shown in SEQ ID NO. 6, and a human PDGF-BB DNA as shown in SEQ ID NO. 7, respectively.
- [0207] No. 38: CD63-TM3-Linker-EGF-T2A-CD63-TM3-Linker-NGF-T2A-CD63-TM3-Linker-PDGF-BB, in which each CD63-TM3 is the TM3 domain of CD63, encoded by DNA sequence shown in SEQ ID NO. 10, each linker is encoded by DNA sequence shown in SEQ ID NO. 12, and each T2A is encoded by DNA sequence shown in SEQ ID NO. 14; EGF, NGF, and PDGF-BB are encoded by a rat EGF DNA as shown in SEQ ID NO. 18, a rat NGF DNA as shown in SEQ ID NO. 19, and a rat PDGF-BB DNA as shown in SEQ ID NO. 20, respectively.
- [0208] No. 39: CD63-TM3-Linker-EGF-T2A-CD63-TM3-Linker-NGF-T2A-CD63-TM3-Linker-PDGF-BB-T2A-CD63-TM3-Linker-IDR-1018, in which each CD63-TM3 is the TM3 domain of CD63, encoded by DNA sequence shown in SEQ ID NO. 10, each linker is encoded by DNA sequence shown in SEQ ID NO. 12, and each T2A is encoded by DNA sequence shown in SEQ ID NO. 14; EGF, NGF, PDGF-BB, and IDR-1018 are encoded by a human EGF DNA as shown in SEQ ID NO. 5, a human NGF DNA as shown in SEQ ID NO. 6, a human PDGF-BB DNA as shown in SEQ ID NO. 7, and an IDR-1018 DNA as shown in SEQ ID NO. 8, respectively.
- [0209] No. 40: CD63-TM3-Linker-EGF-T2A-CD63-TM3-Linker-NGF-T2A-CD63-TM3-Linker-PDGF-BB-T2A-CD63-TM3-Linker-IDR-1018, in which each CD63-TM3 is the TM3 domain of CD63, encoded by DNA sequence shown in SEQ ID NO. 10, each linker is encoded by DNA sequence shown in SEQ ID NO. 12, and each T2A is encoded by DNA sequence shown in SEQ ID NO. 14; EGF, NGF, PDGF-BB and IDR-1018 are encoded by a rat EGF DNA as shown in SEQ ID NO. 18, a rat NGF

DNA as shown in SEQ ID NO. 19, a rat PDGF-BB DNA as shown in SEQ ID NO. 20, and an IDR-1018 DNA as shown in SEQ ID NO. 8, respectively.

[0210] Generation of stable cell lines: The stable cell line, e.g. based on HEK293 cell line, expressing target proteins, including EGF, NGF, PDGF-BB and/or IDR-1018, was generated by transfection with the plasmids or infection with the corresponding lentiviruses. Forty-eight hours after transfection or infection, cells were selected by the addition of blasticidin (Solarbio Life Sciences) to a final concentration of 6 µg/ml. A single cell colony with green fluorescent protein (GFP) expression was selected and cultured in complete medium with 6 µg/ml blasticidin. The stable cell line was monitored for the expression of GFP and the corresponding targeted proteins.

[0211] Adaptation of cell culture to a SFM (serum-free medium) : After three initial passages in FM (full medium) from HEK293 stable cell line, adaptation for serum-free culture was started from the 4th passage. Cells were subcultured with medium composition in Table 1. To establish the fully adapted serum-free culture, cells should be subcultured in a SFM (HyCloneTM peak expression, SH31193.02, Cytiva Life Sciences) for at least three times.

[0212] Table 1. Culture Medium for Adaptation

Steps	Sequential adaptation
0	100% FM
1	75% FM + 25% SFM
2	50% FM + 50% SFM
3	25% FM + 75% SFM
4	12.5% FM + 87.5% SFM
5	5% FM + 95% SFM
6	100% SFM

[0213] FM: Full medium; SFM: Serum-free medium

[0214] Exosomes isolation: The stable cell line was seeded in T150 flasks for 24h, rinsed extensively with PBS and incubated in SFM for another 48h. The cultured cell-free extracellular medium containing exosomes was harvested by centrifugation at 300×g for 10 min to remove the cells. Then centrifuge at 10,000×g for 30 min to remove dead cells and cell debris. Finally, the clear supernatant was centrifuged for 70 min at 100,000×g to pellet the exosomes for twice. And the exosome pellet was resuspended. All centrifugation steps were carried out at 4 °C.

- [0215] Figure 1 shows schematically the flowchart of exosome production process derived from HEK293 cells, according to an exemplary embodiment of the present disclosure.
- [0216] Example 2. Characterization of Exosomes
- [0217] Analysis of the particle concentration and size distribution of exosomes
- [0218] The particle concentration and size distribution of exosomes from stable cell line NO. 38 or NO. 40 (transduced with pGOI No. 38 and 40, respectively) were analyzed by the NanoFCM (NanoFCM Inc., Xiamen, China). The NanoFCM analysis used two single photon counting avalanche photodiodes (APDs) to detect individual particle side scatter (SSC) and fluorescence simultaneously. Firstly, the exosomes pellet was prepared in PBS. Then, 200 nm PE and AF488 fluorophore-conjugated polystyrene beads were used for particle concentration and Silica Nanosphere Cocktail (NanoFCM Inc., Xiamen, China) for particle size distribution. The detector recorded particles passing by during a 1-min interval in each test. Each sample was diluted to reach a particle count within the optimal range of 3000-9,000 particles per minute. NanoFCM software (NanoFCM Profession V2.0) was used to convert flow rate and side scattering intensity to vesicle concentration and size.
- [0219] Figure 2A shows the particle diameter and particle concentration of exosomes derived from the stable cell lines No. 38 and 40 were analyzed by NanoFCM. The exosomes have a mean particle diameter of 70.8 nm for No. 38, and 73.2 nm for No. 40.
- [0220] Identification of target proteins on exosomes via Western blotting (WB) or ELISA
- [0221] For the identification of target proteins expressed on exosomes, the purified exosomes were lysed with RIPA lysis buffer (Beyotime) supplemented with 1 mM protease inhibitor phenylmethylsulfonyl fluoride (PMSF; Beyotime) and phosphatase inhibitor (Beyotime), then heat denatured, separated by SDS-PAGE, and transferred onto PVDF membrane (Millipore, USA). The proteins were detected by incubation with primary antibody against CD63, the fused scaffold protein of exosomes, followed by incubation with a HRP-conjugated secondary antibody (Invitrogen). Enhanced chemiluminescence reagent (Millipore, MA, USA) was then used for the visualization of the membranes. For detection of target proteins concentration, the purified exosomes were prepared and the target proteins on exosomes were analyzed via an appropriate ELISA kit (Solarbio, SEKR-0010; SEKR-0036; SEKH-0290; SEKH-0050) according to manufacturer's instructions.
- [0222] Transmission electron microscopy (TEM) analysis of exosomes
- [0223] TEM was used to confirm the presence of exosomes. Approximately, 20 μ l of exosomes were added separately to copper grids. All excess fluids were removed using filter paper, and the samples were negatively stained with 2% uranyl acetate for 30 s. The grids were rinsed in deionized water and allowed to dry overnight. The samples

were then air-dried using an electric incandescent lamp and viewed using an electron microscope (Hitachi, S-3000N) .

[0224] Figure 2B shows the transmission electron microscopy (TEM) image of the exosomes produced from stable cell lines No. 38 and 40. Figure 2C shows the immunoblotting analysis of exosomes from cell line No. 38 and 40 with antibody against CD63-TM3, the exosome fused scaffold proteins. As expected, the engineered exosome-associated target proteins, NGF, PDGF-BB, EGF were present in purified exosomes derived from both stable cell lines No. 38 and 40. While IDR-1018 were present in purified exosomes derived from stable cell line No. 40.

[0225] The concentration of functional proteins within exosomes were measured via ELISA kit. Table 2 shows the results of concentrations of different proteins as determined by ELISA.

[0226] Table 2. Concentrations of effective factors determined by ELISA

Concentration of effective factors (pg/ml exosomes)				
	Ra-NGF	Ra-EGF	Ra-PDGF-BB	IDR-1018
No.38	ND	85.16	2943.45	ND
	Hu-NGF	Hu-EGF	Hu-PDGF-BB	IDR-1018
No.40	ND	98.84	3078.27	ND

[0227] Example 3. Scratch wounding assay

[0228] HSF (human skin fibroblast) cells were prepared for scratch wounding assay. Once HSF cells were at confluence, scrape cell layer in a straight line using a 1 mm pipette tip. After scratching, gently wash cell monolayer to remove detached cells, then replenish with fresh medium (Mock) or exosomes (No. 1, No. 37, No. 39) , respectively. Then images were captured using a microscope on 10x magnification at different time points (0h, 24h, 48h) .

[0229] Images were shown in Fig. 3, in which the exosomes were produced from stable cell lines No. 1, No. 37 or No. 39. The scratch assay method was used to evaluate the in vitro wound closure effects of engineered exosome derived from stable cell lines No.1, No. 37 or No. 39 on HSF. At 24 hours, there was an obvious wound closure for the treatment with exosome from stable cell lines No. 37 or No. 39 compared with No. 1 or mock treatment. Especially for exosomes from No. 39, the extent of wound closure had the highest value. Furthermore, complete wound closure of the scratches was seen in HSF cultures by 48 hours only when treated with exosomes from No. 39. It suggested that exosomes (No. 39) with four functional proteins have better repair

efficacy for wound healing compared with exosomes (No. 37) with three functional proteins or exosomes (No. 1) with one functional protein.

- [0230] Example 4. Construction of a wound healing model with diabetic foot ulcer (DFU) rat
- [0231] All animal experiments were performed under protocols approved by the Institutional Animal Care and Use Committee of WuXi AppTec (WuXi, China). The experiment was designed following animal welfare guidelines and the three Rs principles. The male Sprague-Dawley (SD) rats (8-week-old) were purchased from Charles River Laboratories (Beijing, China) and housed under specific pathogen-free (SPF) conditions as diabetic wound healing animal models. Each rat was individually caged to prevent biting and fighting with other rats. All rats had free access to water and standard laboratory chow.
- [0232] A single intraperitoneal injection of 60 mg/kg streptozotocin (STZ) was given to induce diabetes in rats. Blood glucose and body weight were measured to confirm whether the diabetic models were successfully established. After 8 weeks, the rats were anesthetized with 3% isoflurane and full-thickness round wounds were created on the left foot.
- [0233] For the wound healing, the rats were randomly divided into four groups: (1) Health-vehicle, in which rats were healthy and wounds healed spontaneously treated with PBS; (2) Model-vehicle, diabetic foot wound models with PBS treatment; (3) NO. 38, diabetic foot wound models with NO. 38 exosome treatment; (4) NO. 40, diabetic foot wound models with NO. 40 exosome treatment. The diabetic models were injected intradermally with 10 μ g exosome in 100 μ l PBS around the wound on day 1, 4, 7, 10, 13, five times in total. Wound kinetics were recorded using a camera each time before administration. The wound area was calculated using an image analysis software (ImageJ) to quantify the wound healing process for evaluating healing efficacy. The calculation was as the following: $\text{Inhibition\%} = 100\% \times (\text{initial wound area} - \text{current wound area}) / \text{initial wound area}$.
- [0234] Fig. 4A shows the schematic diagram of construction of DFU rat model and design of the intradermal administration. Fig. 4B and 4C show the blood glucose and body weight of the animals at different time points.
- [0235] Figure 5 shows the engineered exosomes exhibit enhanced wound healing effect in rat DFU model. Both NO. 38 and 40 exosome treatments significantly improved wound healing rate on day 13, compared to model vehicle. NO. 40 exosome treatment was more effective and significantly superior to NO. 38 exosome and almost comparable to healthy vehicle.
- [0236] The wound tissues of DFU rats were collected at the end of the study. The HE staining within DFU model group (Model-Vehicle) and DFU model treated with

exosome (NO. 40) was analyzed. The vessel numbers were confirmed with CD31, and the nerve repair was shown with PGP9.5 as a detection marker. CD31 is an endothelial cell marker in normal tissues that is secreted by endothelial cells of blood and PGP9.5 is expressed in neurons and neuroendocrine cells, which is used to identify central and peripheral nerves.

- [0237] Results shown in Fig. 6 demonstrated that engineered exosomes promoted blood vessel and nerve repair in rat DFU model. The immunohistochemistry (IHC) results showed a remarkable upward trend of the CD31 (Fig. 6A) and PGP9.5 (Fig. 6D) expressions in diabetic wound bed when treated with exosomes (NO. 40) for 5 times at the end of the study.
- [0238] The Normal injury healing rate over 16 days for each group (n = 6) including the model group (Vehicle) and model treated with exosome (NO. 40) groups was calculated. At the end of study (Day 16), the tissue at the injury sites were prepared for HE staining, and the escharosis and pro-inflammatory cells were analyzed. Data were shown as mean $\pm$ SD. *represents $p < 0.05$ compared with the control group.
- [0239] Results shown in Fig. 7 demonstrated that engineered exosomes promoted normal injury healing at different time points. In the normal injury healing model, the wound healing rate was faster when treated with exosomes (NO. 40) compared to model group (Fig. 7A). The downregulation of pro-inflammatory cells (Figs. 7B&C) suggested that an aggressive pro-inflammatory or "M1" phenotype has been shifted to a pro-healing or "M2" phenotype at the late phase of healing, which can promote the final recovery. The decreased escharosis (Figs. 7B&C) can contribute to removing the obstacles to the healing process and accelerating re-epithelialization.

Claims

- [Claim 1] An engineered exosome, comprising
(a) an EGF polypeptide, fused to a first anchoring polypeptide,
(b) a NGF polypeptide, fused to a second anchoring polypeptide, and
(c) a PDGF-BB polypeptide, fused to a third anchoring polypeptide,
wherein (a) , (b) and (c) are anchored on a membrane of the exosome
via the first, second and third anchoring polypeptide, respectively, and
wherein the EGF polypeptide, the NGF polypeptide and the PDGF-BB
polypeptide are exposed on an outer surface of the membrane of the
exosome.
- [Claim 2] The engineered exosome of claim 1, wherein the engineered exosome
further comprises (d) an IDR-1018 polypeptide comprising the amino
acid sequence shown in SEQ ID NO. 4, fused to a fourth anchoring
polypeptide, and the IDR-1018 polypeptide is exposed on the outer
surface of the membrane of the exosome.
- [Claim 3] The engineered exosome of claim 1, wherein the EGF polypeptide
is a human EGF or an ortholog or paralog thereof, preferably the
EGF polypeptide comprises an amino acid sequence having at least
80%identity to the amino acid sequence as shown in SEQ ID NO. 1.
- [Claim 4] The engineered exosome of claim 1, wherein the NGF polypeptide
is a human NGF or an ortholog or paralog thereof, preferably the
NGF polypeptide comprises an amino acid sequence having at least
80%identity to the amino acid sequence as shown in SEQ ID NO. 2.
- [Claim 5] The engineered exosome of claim 1, wherein the PDGF-BB
polypeptide is a human PDGF-BB dimer or an ortholog or paralog
thereof; more preferably the PGDF-BB dimer comprises an amino acid
sequence having at least 80%identity to the amino acid sequence as
shown in SEQ ID NO. 3.
- [Claim 6] The engineered exosome of claim 1 or 2, wherein the anchoring
polypeptides are membrane proteins of exosome, membrane-targeting
sequences, or an anchoring functional fragment thereof; preferably,
the membrane proteins of exosome include lamp2b, tetraspanins such
as CD63, CD9 and CD81, platelet-derived growth factor receptors
(PDGFRs) , lactadherin (C1C2 domain) , vesicular stomatitis virus
glycoprotein (VSVG) , prostaglandin F2 receptor negative regulator
(PTGFRN) and any combination thereof; preferably the membrane-
targeting sequences include glycosylphosphatidylinositol (GPI) anchors

and lipid-anchored proteins; preferably, the anchoring polypeptides comprise a full-length CD63 or a truncated CD63 that retains TM3 domain; more preferably, each of the first, second, third, and optionally, fourth anchoring polypeptides comprises a TM3 domain of CD63; more preferably, each of the anchoring polypeptides is a TM3 domain of CD63; preferably, the TM3 domain of CD63 comprises an amino acid sequence having at least 80% identity to the amino acid sequence as shown in SEQ ID NO. 9.

- [Claim 7] The engineered exosome of claim 1 or 2, wherein the EGF polypeptide, the NGF polypeptide, the PDGF-BB polypeptide, and the IDR-1018 polypeptide is fused to the C-terminus of the first, second, third, and fourth anchoring polypeptides, respectively, optionally through a peptide linker, preferably, the peptide linker consists of glycine and serine, e.g., (G4S)_n, in which n is an integer from 1 to 3.
- [Claim 8] The engineered exosome of claim 1, wherein the exosome is not derived from a mesenchymal stem cell; preferably, the exosome is not derived from a stem cell.
- [Claim 9] A composition comprising the exosome of any of claims 1 to 8, and a carrier; preferably, the composition is a liquid formulation; more preferably, the composition is formulated for topical or subcutaneous administration; and preferably the composition is a pharmaceutical composition.
- [Claim 10] The composition of claim 9, wherein the composition does not contain an EGF polypeptide (e.g. human EGF polypeptide), a NGF polypeptide (e.g., human NGF polypeptide), a PDGF-BB polypeptide (e.g., a human PDGF-BB polypeptide), or an IDR-1018 polypeptide, not attached to the membrane of the exosome.
- [Claim 11] A nucleic acid construct comprising a polynucleotide encoding (a) an EGF polypeptide fused to a first anchoring polypeptide, (b) a NGF polypeptide fused to a second anchoring polypeptide, and (c) a PDGF-BB polypeptide fused to a third anchoring polypeptide.
- [Claim 12] The nucleic acid construct of claim 11, wherein the polynucleotide further encodes (d) an IDR-1018 polypeptide comprising the amino acid sequence shown in SEQ ID NO. 4 and fused to a fourth anchoring polypeptide.
- [Claim 13] The nucleic acid construct of claim 11 or 12, wherein (i) the EGF polypeptide is a human EGF or an ortholog or paralog thereof, preferably the EGF polypeptide comprises an amino acid

sequence having at least 80% identity to the amino acid sequence as shown in SEQ ID NO. 1;

(ii) the NGF polypeptide is a human NGF or an ortholog or paralog thereof, preferably the NGF polypeptide comprises an amino acid sequence having at least 80% identity to the amino acid sequence as shown in SEQ ID NO. 2;

(iii) the PDGF-BB polypeptide is a human PDGF-BB dimer; more preferably the PDGF-BB dimer comprises an amino acid sequence having at least 80% identity to the amino acid sequence as shown in SEQ ID NO. 3;

(iv) a polynucleotide encoding the EGF polypeptide has a nucleotide sequence as shown in SEQ ID NO. 5 or a degenerate sequence thereof;

(v) a polynucleotide encoding the NGF polypeptide has a nucleotide sequence as shown in SEQ ID NO. 6 or a degenerate sequence thereof;

(vi) a polynucleotide encoding the PDGF-BB polypeptide has a nucleotide sequence as shown in SEQ ID NO. 7 or a degenerate sequence thereof;

(vii) a polynucleotide encoding the IDR-1018 polypeptide has a nucleotide sequence as shown in SEQ ID NO. 8 or a degenerate sequence thereof;

(viii) the anchoring polypeptides are membrane proteins of exosome, membrane-targeting sequences, or an anchoring functional fragment thereof; preferably, the membrane proteins of exosome include lam-p2b, tetraspanins such as CD63, CD9 and CD81, platelet-derived growth factor receptors (PDGFRs), lactadherin (C1C2 domain), vesicular stomatitis virus glycoprotein (VSVG), prostaglandin F2 receptor negative regulator (PTGFRN) and any combination thereof; preferably the membrane-targeting sequences include glycosylphosphatidylinositol (GPI) anchors and lipid-anchored proteins; preferably, the anchoring polypeptides comprise a full-length CD63 or a truncated CD63 that retains TM3 domain; more preferably, each of the first, second, third, and optionally, fourth anchoring polypeptides comprises a TM3 domain of CD63; more preferably, each of the first, second, third, and optionally, fourth anchoring polypeptides is a TM3 domain of CD63; and/or

(ix) a polynucleotide encoding the TM3 domain of CD63 has a nucleotide sequence as shown in SEQ ID NO. 10 or a degenerate sequence thereof.

- [Claim 14] The nucleic acid construct of any of claims 11 to 13, wherein the polynucleotide is a single polynucleotide comprising a fragment of nucleotides encoding the polypeptides (a) , (b) , (c) and, optionally, (d) , with each of the polypeptides (a) , (b) , (c) and, optionally, (d) linked to the anchoring polypeptide; preferably the polypeptides (a) , (b) , (c) and, optionally, (d) are spaced by self-cleavage peptides; optionally, the self-cleavage peptides are 2A peptides, e.g., T2A, E2A, P2A or any combination thereof.
- [Claim 15] A vector comprising the nucleic acid construct of any of claims 11 to 14; preferably, the vector is a viral vector; more preferably the vector is a lentiviral vector or an adeno-associated viral vector.
- [Claim 16] A cell transduced with the vector of claim 15, wherein the polynucleotide is integrated into the genome of the cell; preferably, the cell is not a mesenchymal stem cell; preferably, the cell is not a stem cell; preferably, the cell is a mammalian cell; or more preferably, the cell is a HEK293 or CHO cell.
- [Claim 17] A method of producing an engineered exosome of any of claims 1 to 8, comprising
(a) transducing the cell of claim 16 with the vector of claim 15;
(b) culturing the cell in a condition allowing secretion of an exosome from the cell; and
(c) collecting and purifying the exosome.
- [Claim 18] The method of claim 17, further comprising adapting the cell to a serum-free condition during step (b) .
- [Claim 19] Use of the engineered exosome of any of claims 1 to 8 or the composition of claims 9 or 10 in manufacturing a medicament for improving wound healing or for treating wound, or for treating Cutibacterium acnes infection; preferably, the wound is a chronic wound, preferably a diabetic wound; more preferably, the wound includes diabetic foot ulcer; preferably the Cutibacterium acnes infection occurs in skin; preferably, the Cutibacterium acnes infection is acnes or acnes skin such as a scar or cutaneous damage caused by acnes.