SUMMARY

[0007] Stem cells modified to express therapeutic genes, or other genes of interest, are desirable for a number of different therapeutic and non-therapeutic applications. Traditionally, in the field of prodrug gene therapy, virus-based gene modification approaches have been the favoured approach for modifying stem cells such as MSCs in preclinical and clinical studies, since non-viral approaches have generally provided poor transfection efficiency. However, virus-based gene modification in such applications has inherent safety risk, production of clinical grade virus can be laborious, and the number of gene copies which may be introduced per cell through viral methods is generally low (often < 10 copies per cell). Furthermore, achieving gene modification of stem cells such as MSCs, either virally or non-virally, without causing undesirable changes to phenotype (i.e. multipotency, immunophenotype, tropism, etc.) of the resultant cells is another challenge facing the field.

[0008] As described in detail herein, the present inventors have now developed methods for transfecting mesenchymal stem cells with a nucleic acid construct from which one or more functional genes are expressed, which are non-viral and which in certain examples may provide high transfection efficiency, high copy number per cell, high cell viability, transient expression for extended duration, and/or a substantially unchanged multipotent phenotype. In certain examples, such methods may be scalable and/or suitable for large scale clinical production of modified mesenchymal stem cells. Also described in detail herein are transfected mesenchymal stem cells and populations of mesenchymal stem cells, uses thereof, methods for the treatment of diseases or disorders such as cancer using such transfected stem cells, and kits and compositions relating thereto.

[0009] In one ~~aspect~~example, the present disclosure refers to a mesenchymal stem cell (MSC) transfected with a nucleic acid construct from which one or more functional genes are expressed, the MSC having a multipotent phenotype which is substantially unchanged by the transfection of the nucleic acid construct, and the MSC being free of virus-based transfection vehicle materials.

[0010] In another ~~aspect~~example, the present disclosure refers to a plurality of mesenchymal stem cells (MSCs), wherein at least about 60% of the MSCs are transfected with a nucleic acid construct

In another example the phenotype may comprise an immunophenotype in which the expression of CD surface markers may be substantially unchanged after transfection.

[0016] In another example of any of the MSC or MSCs described herein, the transfected MSC or MSCs may be plastic-adherent, may express CD105, CD73, and CD90 (>95%), may lack expression of CD45, CD34, CD14, and HLA-DR surface molecules (<2%), and may be capable of differentiating into osteoblasts, adipocytes, and chondroblasts *in vitro*, satisfying the immunophenotype criteria defined by the International Society for Cellular Therapy (ISCT). In another example, the transfected MSC or MSCs may be undifferentiated.

[0017] In another example of any of the MSC or MSCs described herein, the MSC or MSCs may be in a cryopreserved state.

[0018] In another example, the MSC or MSCs may be for use in treating cancer. In certain examples, the cancer may comprise lymphoma, clear cell carcinoma, glioblastoma, temozolomide resistant glioblastoma, perianal carcinoma, oral melanoma, thyroid carcinoma, soft tissue carcinoma, cancer ulceration, nasal tumor, or gastrointestinal cancer, or any combination thereof. In another example, the MSC or MSCs may be for use in combination with 5FC, 5FU, GCV, or any combination thereof.

[0018a] In another ~~aspect~~example, the present disclosure refers to use of the MSC or MSCs as disclosed herein, in the manufacture of a medicament for treating cancer, for example lymphoma, clear cell carcinoma, glioblastoma, temozolomide resistant glioblastoma, perianal carcinoma, oral melanoma, thyroid carcinoma, soft tissue carcinoma, cancer ulceration, nasal tumor, or gastrointestinal cancer.

[0018b] In another ~~aspect~~example, the present disclosure refers to the MSC or MSCs as disclosed herein for use as a medicament.

[0019] In another ~~aspect~~example, the present disclosure refers to a method for transfecting mesenchymal stem cells (MSCs) with a nucleic acid construct from which one or more functional genes are expressed, the method comprising: exposing the MSCs to a transfection mixture comprising the nucleic acid construct which is complexed with a cationic polymer; exposing the MSCs to a first agent capable of redirecting endocytosed nucleic acids from intracellular acidic compartments and a second agent capable of stabilizing a microtubular network of the MSCs; and incubating the MSCs; thereby providing MSCs transfected with the nucleic acid construct.

[0019a] In one aspect, the present disclosure refers to a method for transfecting mesenchymal stem cells (MSCs) with a nucleic acid construct from which one or more functional genes are expressed, the method comprising:

exposing the MSCs to a transfection mixture comprising the nucleic acid construct which is complexed with a cationic polymer;

exposing the MSCs to a first agent capable of redirecting endocytosed nucleic acids from intracellular acidic compartments and a second agent capable of stabilizing a microtubular network of the MSCs; and

incubating the MSCs;

wherein the MSCs are not centrifuged during exposure to the transfection mixture, to the first agent and second agent, during incubation, or any combination thereof;

thereby providing MSCs transfected with the nucleic acid construct.

[0020] In another example of and of the method or methods described herein the MSCs may not be centrifuged during exposure to the transfection mixture, to the first agent and second agent, during incubation, or any combination thereof. In another example the step of incubating the MSCs may comprise gentle mixing without centrifugation. In another example the step of incubating the MSCs may comprise incubating the MSCs for at least

[0043] In another example there is provided herein a composition comprising the engineered MSC or MSCs of any example described herein, and at least one of a pharmaceutically acceptable carrier, diluent, excipient, cell media, or buffer.

[0043a] In another ~~aspect~~example, the present disclosure refers to a pharmaceutical composition comprising the MSC or MSCs as disclosed herein, and at least one of a pharmaceutically acceptable carrier, diluent, excipient, cell media, or buffer.

[0044] In another example there is provided herein a theranostic agent comprising any of the MSC or MSCs of any example described herein.

[0045] In another example there is provided herein a kit for transfecting a mesenchymal stem cell (MSC) with a nucleic acid construct from which one or more functional genes are transiently expressed. In an example the kit may comprise one or more of: an MSC; a nucleic acid construct designed for transient expression of one or more functional genes; a cell culture media; a cationic polymer; a first agent capable of redirecting endocytosed nucleic acids from intracellular acidic compartments; a second agent capable of stabilizing a microtubular network of the MSC; instructions for performing a method as described in any example herein; 5FC; GCV; and/or 5FU. In certain examples, the kit may comprise a cryopreservation buffer or agent, a thawing buffer or agent, or both. In certain non limiting examples, cryopreservation buffers or solutions can be used, such as cryostor10 (Biolife Solutions USA). In further exemplary examples, thawed engineered MSCs can be stored in a hypothermic solution such as Hypothermosol (Biolife Solutions USA). Other examples may also be used as will be apparent to the skilled person in the art.

[0045a] In another ~~aspect~~example, the present disclosure refers to a kit for transfecting a mesenchymal stem cell (MSC) with a nucleic acid construct from which one or more functional genes are transiently expressed, the kit comprising one or more of:

an MSC; a nucleic acid construct designed for transient expression of one or more functional genes; a cell culture media; a cationic polymer;

a first agent capable of redirecting endocytosed nucleic acids from intracellular acidic compartments; a second agent capable of stabilizing a microtubular network of the MSC;

instructions for performing a method as disclosed herein;

5FC;

GCV; and/or

5FU.

[0046] In another example of any of the kit or kits herein, the MSC may be derived from cord blood, neonatal birth-associated tissue, Wharton's jelly, umbilical cord, cord lining, placenta, or other source of MSC cells. In another example the MSC may be an adipose tissue-derived MSC (AT-MSC), bone marrow-derived MSC (BM-MSC), or umbilical cord-derived MSC (UC-MSC). In another example, the MSCs may be sourced from human, canine, feline, equine, or other species. In another example the nucleic acid construct may comprise a CpG-free expression plasmid or other CpG-free expression construct, a scaffold/matrix attachment region (S/MAR), an episomal vector, or an EBNA-1 containing construct. In another example the cationic polymer may comprise linear or branched polyethylenimine (PEI), poly(amidoamine) PAMAM, or another cationic polymer, or any combinations thereof. In another example the cationic polymer may comprise linear

WHAT IS CLAIMED IS:

1. A mesenchymal stem cell (MSC) transfected with a nucleic acid construct from which one or more functional genes are expressed, the MSC having a multipotent phenotype which is substantially unchanged by the transfection of the nucleic acid construct, and the MSC being free of virus-based transfection vehicle materials.

2. A plurality of mesenchymal stem cells (MSCs), wherein at least about 60% of the MSCs are transfected with a nucleic acid construct from which one or more functional genes are expressed, the transfected MSCs having a multipotent phenotype which is substantially unchanged by the transfection of the nucleic acid construct, and the MSCs being free of virus-based transfection vehicle materials.

3. The MSC or MSCs of claim 1 or 2, wherein the transfected MSC or MSCs are each transfected with an average of at least about 1000, at least about 2000, at least about 3000, at least about 4000, at least about 5000, at least about 6000, at least about 7000, at least about 8000, at least about 9000, or at least about 10000 copies of the nucleic acid construct;

wherein optionally the transfected MSC or MSCs transiently express the one or more functional genes for at least about 7, at least about 8, at least about 9, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, or at least about 17 days following transfection;

wherein optionally the transfected MSC or MSCs are transfected with the nucleic acid construct using a cationic polymer, a first agent capable of redirecting endocytosed nucleic acids from intracellular acidic compartments, and a second agent capable of stabilizing a microtubular network of the MSC or MSCs;

wherein optionally the one or more functional genes comprise a suicide gene;

~~wherein optionally the one or more functional genes comprise Cytosine Deaminase (CDy), uracil phosphoribosyltransferase (UPRT), or both; and~~

~~wherein optionally the multipotent phenotype includes tumor and/or cancer tropism properties of the MSC.~~

~~4. The MSC or MSCs of any one of claims 1 – 3, wherein the multipotent phenotype comprises an immunophenotype in which the expression of CD surface markers is substantially unchanged after transfection, preferably wherein the transfected MSC or MSCs are plastic-adherent, express CD105, CD73, and CD90 (>95%), lack expression of CD45, CD34, CD14, and HLA-DR surface molecules (<2%), and are capable of differentiating into osteoblasts, adipocytes, and chondroblasts *in vitro*, satisfying the immunophenotype criteria defined by the International Society for Cellular Therapy (ISCT).~~

~~5. Use of the MSC or MSCs of any one of claims 1-4, in the manufacture of a medicament for treating cancer, for example lymphoma, clear cell carcinoma, glioblastoma, temozolomide resistant glioblastoma, perianal carcinoma, oral melanoma, thyroid carcinoma, soft tissue carcinoma, cancer ulceration, nasal tumor, or gastrointestinal cancer.~~

~~6. The use according to claim 5, wherein the MSC or MSCs are to be administered in combination with 5FC, 5FU, GCV, or any combination thereof.~~

~~7.~~ 1. A method for transfecting mesenchymal stem cells (MSCs) with a nucleic acid construct from which one or more functional genes are expressed, the method comprising:

exposing the MSCs to a transfection mixture comprising the nucleic acid construct which is complexed with a cationic polymer;

exposing the MSCs to a first agent capable of redirecting endocytosed nucleic acids from intracellular acidic compartments and a second agent capable of stabilizing a microtubular network of the MSCs; and

incubating the MSCs;

wherein the MSCs are not centrifuged during exposure to the transfection mixture, to the first agent and second agent, during incubation, or any combination thereof;

thereby providing MSCs transfected with the nucleic acid construct.

~~8.~~ 2. The method of claim ~~7~~1, ~~wherein the MSCs are not centrifuged during exposure to the transfection mixture, to the first agent and second agent, during incubation, or any combination thereof;~~

wherein optionally the step of incubating the MSCs comprises incubating the MSCs for about 2 hours to about 48 hours;

~~wherein optionally the step of exposing the MSCs to the first and second agents comprises replacing the transfection mixture with cell culture media supplemented with the first agent and second agent;~~

wherein optionally the one or more functional genes comprise a suicide gene;

wherein optionally the one or more functional genes comprise Cytosine Deaminase (CDy), uracil phosphoribosyltransferase (UPRT), or both;

wherein optionally the transfected MSCs are each transfected with an average of at least about 1000, at least about 2000, at least about 3000, at least about 4000, at least about 5000, at least about 6000, at least about 7000, at least about 8000, at least about 9000, or at least about 10000 copies of the nucleic acid construct;

wherein optionally a multipotent phenotype of the transfected MSCs is substantially unchanged by the transfection,

wherein optionally the multipotent phenotype comprises tumor and/or cancer tropism properties of the MSC; and

wherein optionally the multipotent phenotype comprises an immunophenotype in which the expression of CD surface markers is substantially unchanged after transfection, preferably wherein the transfected MSCs are plastic-adherent, wherein >95% of the transfected MSCs express CD105, CD73, and CD90 ~~(>95%)~~, wherein <2% of the transfected MSCs ~~lack~~ expressi~~on of~~ CD45, CD34, CD14, and HLA-DR surface molecules ~~(<2%)~~, and wherein the transfected MSCs are capable of differentiating into osteoblasts, adipocytes, and chondroblasts *in vitro*~~, satisfying the immunophenotype criteria defined by the International Society for Cellular Therapy (ISCT)~~.

~~9. A pharmaceutical composition comprising the MSC or MSCs of any one of claims 1-4, and at least one of a pharmaceutically acceptable carrier, diluent, excipient, cell media, or buffer.~~

~~10. A kit for transfecting a mesenchymal stem cell (MSC) with a nucleic acid construct from which one or more functional genes are transiently expressed, the kit comprising one or more of:~~

~~an MSC;~~

~~a nucleic acid construct designed for transient expression of one or more functional genes;~~

~~a cell culture media;~~

~~a cationic polymer;~~

~~a first agent capable of redirecting endocytosed nucleic acids from intracellular acidic compartments;~~

~~a second agent capable of stabilizing a microtubular network of the MSC;~~

~~instructions for performing a method as defined in claim 7 or 8;~~

~~5FC;~~

~~GCV; and/or~~

~~5FU.~~

3. The method of claim 1 or 2, wherein the step of exposing the MSCs to the first and second agents comprises replacing the transfection mixture with cell culture media supplemented with the first agent and second agent.

~~11.~~ ~~4.~~ The method of any one of claims ~~7 or 8~~1-3, wherein the step of exposing the MSCs to the transfection mixture comprises adding the transfection mixture to the MSCs without removing a growth medium from the MSCs, and centrifugation is not performed during the steps of exposing and incubating.

~~12.~~ ~~5.~~ The method of any one of claims ~~7, 8 or 11~~1, 2 and 4, wherein the step of exposing the MSCs to the first agent and the second agent comprises adding the first and second agent to the MSCs simultaneously, sequentially, or in combination with the transfection mixture.~~;~~

~~wherein optionally the first and second agent are added to the MSCs simultaneously with addition of the transfection mixture to the MSCs, or wherein the first and second agent are mixed with the transfection mixture and added to the MSCs, or~~

~~wherein the first and second agent are added to the MSCs shortly after the transfection mixture is added to the MSCs.~~

~~13. The method of claim 12, wherein the transfection mixture is not removed before the first and second agents are added to the MSCs.~~

~~14.~~ ~~6.~~ The method of any one of claims ~~7, 8 or 11-13~~1, 2, 4 and 5, wherein a duration of exposure of the MSCs to the transfection mixture overlaps with a duration of exposure of the MSCs to the first and second agents, preferably wherein the transfection mixture is not removed before the first and second agents are added to the MSCs.

~~15.~~ 7. A method for transfecting mesenchymal stem cells (MSCs) with a nucleic acid construct from which one or more functional genes are expressed, the method comprising:

culturing the MSCs in a growth medium;

adding a transfection mixture comprising the nucleic acid construct which is complexed with a cationic polymer to the MSCs without removing the growth medium from the MSCs;

adding a first agent capable of redirecting endocytosed nucleic acids from intracellular acidic compartments and a second agent capable of stabilizing a microtubular network of the MSCs to the MSCs; and

incubating the MSCs while in contact with all of the transfection mixture, the first agent, and the second agent for an incubation period;

wherein the first and second agents are added to the MSCs simultaneously with the addition of the transfection mixture, sequentially with the addition of the transfection mixture, or in combination with the transfection mixture; and

wherein the MSCs are not centrifuged between the adding of the transfection mixture and expiry of the incubation period;

thereby providing MSCs transfected with the nucleic acid construct.

~~16. The MSC or MSCs of any one of claims 1 – 4 for use as a medicament.~~

9. The method of any one of claims 1-8, wherein the MSCs are transfected with the nucleic acid construct from which one or more functional genes are expressed, the MSCs having a multipotent phenotype which is substantially unchanged by the transfection of the nucleic acid construct, and the MSCs being free of virus-based transfection vehicle materials.

10. The method of any one of claims 1-9, wherein the transfected MSCs transiently express the one or more functional genes for at least about 7, at least about 8, at least about 9, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, or at least about 17 days following transfection.

11. The method of any one of claims 1-10, wherein the transfected MSCs are for use in treating cancer, wherein the cancer is lymphoma, clear cell carcinoma, glioblastoma, temozolomide resistant glioblastoma, perianal carcinoma, oral melanoma, thyroid carcinoma, soft tissue carcinoma, cancer ulceration, nasal tumor, or gastrointestinal cancer.

12. The method of any one of claims 1-11, wherein the MSCs are for use in combination with 5-fluorocytosine (5FC), 5-fluorouracil (5FU), ganciclovir (GCV), or any combination thereof.