Summary

In one aspect of the present disclosure, there is provided a method for reprogramming a source cell to produce a cell that exhibits at least one characteristic of a chondrocyte comprising:

i) providing a source cell, or a cell population comprising a source cell;

ii) contacting said source cell with one or more agents that activate or increase the expression of two or more transcription factors; and

iii) culturing said cell or cell population, and optionally monitoring the cell or cell population for at least one characteristic of a chondrocyte cell, wherein:

- the transcription factors are two or more of those listed in Tables 1 to 4.

In another aspect of the present disclosure, there is provided a method of generating a cell exhibiting at least one characteristic of a chondrocyte from a de-differentiated chondrocyte, the method comprising:

- increasing the amount of two or more transcription factors, or variants thereof, in a de-differentiated chondrocyte; and
- culturing the de-differentiated chondrocyte for a sufficient time and under conditions to allow re-differentiation to a chondrocyte, wherein:
- the transcription factors are two or more of those listed in Table 3.

In a further aspect of the present disclosure, there is provided a method of preventing the de-differentiation of a chondrocyte cell culture, the method comprising:

- providing a de-differentiated chondrocyte cell, or a cell population comprising a de-differentiated chondrocyte cell;
- contacting said de-differentiated chondrocyte cell with one or more agents that activate or increase the expression of two or more transcription factors; and

- culturing said de-differentiated cell or cell population, and optionally monitoring the cell or cell population for at least one characteristic of a chondrocyte cell thereby preventing the de-differentiation of the chondrocyte and maintaining at least one characteristic of a chondrocyte in the chondrocyte cell culture,

- wherein the transcription factors are two or more of those listed in Table 3.

In another aspect of the present disclosure, there is provided a cell exhibiting at least one characteristic of a chondrocyte, wherein the cell is produced by a method disclosed herein.

In a further aspect of the present disclosure, there is provided a population of cells, wherein at least 5% of cells exhibit at least one characteristic of a chondrocyte, wherein the cells are produced by a method disclosed herein.

In another aspect of the present disclosure, there is provided a population of cells disclosed herein, for use in treating osteoarthritis or other condition characterised by degeneration of cartilage tissue.

In a further aspect of the present disclosure, there is provided one or more of [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2- naphthalenyl)carboxamido]benzoic acid (AM580), all-trans retinoic acid, 9-cis retinoic acid, beta-estradiol, calcitriol, ciglitazone, kartogenin, lithium chloride, melatonin, rhosin hydrochloride, leucovorin, vorinostat and forskolin, for use in treating osteoarthritis or other condition characterised by degeneration of cartilage tissue.

In another aspect of the present disclosure, there is provided a composition comprising at least one chondrocyte and at least one agent which increases the activity or the protein expression of two or more transcription factors in the chondrocyte, wherein the transcription factor may be any of the transcription factors listed in Tables 1 to 4.

The present disclosure provides a method for reprogramming a source cell, the method comprising increasing the protein expression of one or more transcription factors, or variants thereof, in the source cell, wherein the source cell is reprogrammed to exhibit at least one characteristic of a chondrocyte, wherein:

- the source cell is selected from the group consisting of dermal fibroblasts embryonic stem cells, or de-differentiated chondrocytes, and

- the transcription factors are one or more of those listed in Tables 1 to 4.

The present disclosure provides a method of generating a cell exhibiting at least one characteristic of a chondrocyte from a source cell, the method comprising:

- increasing the amount of one or more transcription factors, or variants thereof, in the source cell; and

- culturing the source cell for a sufficient time and under conditions to allow differentiation to a chondrocyte; thereby generating the cell exhibiting at least one characteristic of a chondrocyte from a source cell, wherein:

- the source cell is selected from the group consisting of dermal fibroblasts, embryonic stem cells, and de-differentiated chondrocytes; and

- the transcription factors are one or more of those listed in Table 1 to 4.

The present disclosure also provides a method for reprogramming a dermal fibroblast, embryonic stem cell, or de-differentiated chondrocyte, the method comprising increasing the protein expression of one or more of the transcription factors in Tables 1 to 4 or variants thereof, in the source cell, wherein the source is reprogrammed to exhibit at least one characteristic of chondrocyte.

The present disclosure provides a method for reprogramming a source cell to a cell that exhibits at least one characteristic of a chondrocyte comprising: i) providing a source cell, or a cell population comprising a source cell; ii) contacting said source cell with one or more agents that activate or increase the expression of one or more transcription factors; and iii) culturing said cell or cell population, and optionally

monitoring the cell or cell population for at least one characteristic of a chondrocyte cell, wherein:

- the source cell is selected from the group consisting of dermal fibroblasts, embryonic stem cells and de-differentiated chondrocytes;

- the transcription factors are one or more of those listed in Tables 1 to 4.

The present disclosure provides a method for reprogramming a source cell to a cell that exhibits at least one characteristic of a chondrocyte comprising: i) providing a source cell, or a cell population comprising a source cell; ii) contacting said source cell with one or more small molecules that activate or increase the expression of one or more transcription factors; and iii) culturing said cell or cell population, and optionally monitoring the cell or cell population for at least one characteristic of a chondrocyte cell, wherein:

- the source cell is selected from the group consisting of dermal fibroblasts, embryonic stem cells and de-differentiated chondrocytes;

- the transcription factors are one or more of those listed in Tables 1 to 4.

Preferably, the transcription factors are one or more of SOX9, PPAR γ, BMP-2, PITX1, TGFβ3, VDR and RUNX1. More preferably, all transcription factors in the group of SOX9, PPAR γ, BMP-2, PITX1, TGFβ3, VDR and RUNX1 are activated or have increase expression resulting from the contact of the source cell with one or more small molecules.

Preferably, the small molecules for activating or increasing transcription of the transcription factors are one or more of [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)carboxamido]benzoic acid (AM580), beta-estradiol, calcitriol, ciglitazone, kartogenin, Lithium chloride, melatonin, and rhosin hydrochloride. More preferably, all 8 small molecules are used.

The present disclosure provides a method for reprogramming a source cell to a cell that exhibits at least one characteristic of a chondrocyte comprising: i) providing a source cell, or a cell population comprising a source cell; ii) transfecting said source cell with one or more nucleic acids comprising a nucleotide sequence that encodes one or

more transcription factors; and iii) culturing said cell or cell population, and optionally monitoring the cell or cell population for at least one characteristic of a chondrocyte cell, wherein:

- the source cell is selected from the group consisting of dermal fibroblasts, embryonic stem cells and de-differentiated chondrocytes;

- the transcription factors are one or more of those listed in Tables 1 to 4.

In any method of the present disclosure described herein, the source cell is a fibroblast, and the target cell is a chondrocyte cell and the transcription factors are any one or more of the transcription factors listed in Tables 1 or 4.

In a preferred example, all of the transcription factors listed in a single row of Table 1 may be used. Alternatively, the factors may be the combination of the transcription factors and proteins listed as follows:

a) PPRX2, PITX1, SMAD6, FOXC1, SIX2, AHR, FOSB and JUNB;

b) BARX1, PPRX2, SMAD6, FOXC1, SIX2, AHR, FOSB and JUNB;

c) BARX1, PITX1, PPRX2, FOXC1, SIX2, AHR, FOSB and JUNB;

d) BARX1, PITX1, SMAD6, PPRX2, SIX2, AHR, FOSB and JUNB;

e) BARX1, PITX1, SMAD6, FOXC1, PPRX2, AHR, FOSB and JUNB;

f) BARX1, PITX1, SMAD6, FOXC1, SIX2, PPRX2, FOSB and JUNB;

g) BARX1, PITX1, SMAD6, FOXC1, SIX2, AHR, PPRX2 and JUNB; or

h) BARX1, PITX1, SMAD6, FOXC1, SIX2, AHR, FOSB and PPRX2.

Preferably, the fibroblast is a dermal fibroblast.

In any method of the present disclosure described herein, the source cell is an embryonic stem cell, and the target cell is a chondrocyte cell and the transcription factors are any one or more of the transcription factors listed in Table 2.

In a preferred example, all of the transcription factors listed in a single row of Table 2 may be used. Alternatively, the factors may be the combination of the transcription factors and proteins listed as follows:

a) HOXA11, PITX1, SMAD6, NFKB1, FOXC1, AHR, SIX2 and JUNB;

b) BARX1, PRRX2, SMAD6, NFKB1, FOXC1, AHR, SIX2 and JUNB;

c) BARX1, PITX1, TGFB3, NFKB1, FOXC1, AHR, SIX2 and JUNB;

d) BARX1, PITX1, SMAD6, IRF1, FOXC1, AHR, SIX2 and JUNB;

e) BARX1, PITX1, SMAD6, NFKB1, PRRX2, AHR, SIX2 and JUNB;

f) BARX1, PITX1, SMAD6, NFKB1, FOXC1, PRRX2, SIX2 and JUNB;

g) BARX1, PITX1, SMAD6, NFKB1, FOXC1, AHR, PRRX2 and JUNB; or

h) BARX1, PITX1, SMAD6, NFKB1, FOXC1, AHR, SIX2 and FOSB.

Preferably, the embryonic stem cell is a human embryonic stem cell.

In any method of the present disclosure described herein, the source cell is a de-differentiated chondrocyte, and the target cell is a chondrocyte cell and the transcription factors are any one or more of the transcription factors listed in Table 3.

In a preferred example, all of the transcription factors listed in a single row of Table 3 may be used. Alternatively, the factors may be the combination of the transcription factors and proteins listed as follows:

a) IL11, SOX5, VEGFA, RUNX1, VDR, NR2F1, FOSB and PRRX1;

b) SOX9, TCF4, VEGFA, RUNX1, VDR, NR2F1, FOSB and PRRX1;

c) SOX9, SOX5, HOXA7, RUNX1, VDR, NR2F1, FOSB and PRRX1;

d) SOX9, SOX5, VEGFA, TCF4, VDR, NR2F1; FOSB and PRRX1;

e) SOX9, SOX5, VEGFA, RUNX1, TCF4, NR2F1, FOSB and PRRX1;

f) SOX9, SOX5, VEGFA, RUNX1, VDR, HOXA7, FOSB and PRRX1;

g) SOX9, SOX5, VEGFA, RUNX1, VDR, NR2F2, FOSB and PRRX1;

h) SOX9, SOX5, VEGFA, RUNX1, VDR, NR2F1, HOXA7 and PRRX1; or

i) SOX9, SOX5, VEGFA, RUNX1, VDR, NR2F1, FOSB and HOXA7.

Preferably, the at least one characteristic of the chondrocyte is up-regulation of any one or more target cell markers and/or change in cell morphology. Relevant markers are described herein and known to those in the art. Exemplary markers for chondrocytes include: CD44, CD49, CD10, CD9, CD95, Integrin α10β1, CD105, SOX9, SMAD3, SOX5, SOX6, SMAD 5/6, the production of sulphated glycosaminoglycans (GAG), aggrecan, type II collagen (expression of COL2A1).

Typically, conditions suitable for chondrocyte differentiation include culturing the cells for a sufficient time and in a suitable medium. A sufficient time of culturing may be at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 days. A suitable medium may be one shown in Table 6.

In any method described herein, the method may further include the step of expanding the cells exhibiting at least one characteristic of a chondrocyte to increase the proportion of cells in a population exhibiting at least one characteristic of a chondrocyte. The step of expanding the cells may be in culture for a sufficient time and under conditions for generating a population of cells as described below.

In any method described herein, the method may further include the step of administering the cells, or cell population including a cell, exhibiting at least one characteristic of a chondrocyte, to an individual.

The present disclosure also provides a method for preventing the de-differentiation of a primary chondrocyte. It will be understood that the approach for preventing de-differentiation mirrors the approach for reprogramming a de-differentiated cell to a re-differentiated chondrocyte. As such, any other methods described herein for re-programming a de-differentiated chondrocyte to a re-differentiated chondrocyte can also be used for preventing de-differentiation of primary chondrocytes.

The present disclosure also provides a cell exhibiting at least one characteristic of a chondrocyte produced by a method as described herein. The cell may be an isolated cell.

The present disclosure also provides a population of cells, wherein at least 5% of cells exhibit at least one characteristic of a chondrocyte and those cells are produced by a method as described herein. Preferably, at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of the cells in the population exhibit at least one characteristic of a chondrocyte. The population of cells may be an isolated population, or substantially pure population of cells.

The present disclosure also relates to kits for producing a cell exhibiting at least one characteristic of a chondrocyte as disclosed herein. In some examples, a kit comprises one or more nucleic acids having one or more nucleic acid sequences encoding a transcription factor described herein or variant thereof.

In alternative examples, a kit comprises one or more small molecules for activating or increasing the expression of one or more transcription factors described herein, or variants thereof. More preferably, the kit comprises one or more of the following small molecules: [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)carboxamido]benzoic acid (AM580), beta-estradiol, calcitriol, ciglitazone, kartogenin, lithium chloride, melatonin, and rhosin hydrochloride.

Preferably, the kit can be used to produce a cell exhibiting at least one characteristic of a chondrocyte. Preferably, the kit can be used with a source cell which is a dermal fibroblast, an embryonic stem cell or a de-differentiated chondrocyte. In some examples, the kit further comprises instructions for reprogramming a source cell to a cell exhibiting at least one characteristic of a chondrocyte according to the methods as disclosed herein. Preferably, the present disclosure provides a kit when used in a method of the present disclosure described herein.

The present disclosure relates to a composition comprising at least one chondrocyte and at least one agent which increases the activity or the protein expression of one or more transcription factors in the chondrocyte. Further, the

transcription factor may be any of the transcription factors described herein. Preferably, the transcription factors are as described in Tables 1 to 4.

In any example of the present disclosure, the agent may be one or more of [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl) carboxamido]benzoic acid (AM580), all-trans retinoic acid, 9-cis retinoic acid, beta-estradiol, calcitriol, ciglitazone, kartogenin, lithium chloride, melatonin, rhosin hydrochloride, leucovorin and forskolin.

In any example, a histone deacetylase inhibitor (HDAC inhibitor), or any other molecule for opening the chromatin in the source cell may be used to increase the efficacy of the small molecule agent. In one example, the molecule is the HDAC inhibitor vorinostat.

Typically, the protein expression, or amount, of a transcription factor as described herein is increased by contacting the cell with an agent which activates or increases the expression of the transcription factor. In any example, the agent is selected from the group consisting of: a nucleotide sequence, a protein, an aptamer and small molecule, ribosome, RNAi agent and peptide-nucleic acid (PNA) and analogues or variants thereof. In certain examples, the agent is exogenous. In preferred examples, the agent is a small molecule. In certain examples, the nucleotide sequence is included as part of a transcriptional activation system (e.g., a gRNA for use in CRISPR/Cas9 systems or a TALEN) for increasing the expression of one or more transcription factors.

In some examples, the small molecule agent is selected from: [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)carboxamido]benzoic acid (AM580), all-trans retinoic acid, 9-cis retinoic acid, beta-estradiol, calcitriol, ciglitazone, kartogenin, lithium chloride, melatonin, rhosin hydrochloride, leucovorin and forskolin.

Preferably, the small molecule agent includes a retinoic acid or an analog or derivative thereof, including any one or more of: [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)carboxamido]benzoic acid (AM580), all-trans retinoic acid and 9-cis retinoic acid.

In some examples, a combination of small molecules is used for increasing the protein expression, or amount of, one or more transcription factors as described herein. For example, in certain examples, the combination of small molecules

includes at least a retinoic acid or an analog or derivative thereof and calcitriol. Alternatively, the combination of small molecules includes at least [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl) carboxamido] benzoic acid (AM580), calcitriol, leucovorin, vorinostat and forskolin. Alternatively, the combination of small molecules may include at least (all-trans)- retinoic acid, 9-cis retinoic acid, calcitriol, leucovorin.

In certain preferred examples, the combination of small molecules is: [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)carboxamido]benzoic acid (AM580), beta-estradiol, calcitriol, ciglitazone, kartogenin, lithium chloride, melatonin, and rhosin hydrochloride. Alternatively, the combination of small molecules is: [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)carboxamido]benzoic acid (AM580), calcitriol, leucovorin, forskolin and vorinostat. Alternatively, the combination of small molecules is: all-trans retinoic acid, 9-cis retinoic acid, calcitriol, leucovorin and vorinostat.

In some examples, the protein expression, or amount, of a transcription factor as described herein is increased by introducing at least one nucleic acid comprising a nucleotide sequence encoding a transcription factor, or encoding a functional fragment thereof, in the cell. Preferably, the nucleotide sequence encoding a transcription factor is at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a sequence with an accession number listed in Table 3.

Preferably, the nucleic acid further includes a heterologous promoter. Preferably, the nucleic acid is in a vector, such as a viral vector or a non- viral vector. Preferably, the vector is a viral vector comprising a genome that does not integrate into the host cell genome. The viral vector may be a retroviral vector or a lentiviral vector.

Any method as described herein may have one or more, or all, steps performed *in vitro*, *ex vivo* or *in vivo*.

In further examples, the present disclosure provides one or more of [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl) carboxamido]benzoic acid (AM580), all-trans retinoic acid, 9-cis retinoic acid, beta-estradiol, calcitriol, ciglitazone, kartogenin, lithium chloride, melatonin, rhosin hydrochloride, leucovorin, vorinostat and

forskolin, for use in a method of treating osteoarthritis or other condition characterised by degeneration of cartilage tissue. Preferably, the present disclosure comprises at least a retinoic acid or derivative thereof (such as [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl) carboxamido]benzoic acid (AM580), all-trans retinoic acid, 9-cis retinoic acid), for use in a method of treating osteoarthritis or other condition characterised by degeneration of cartilage tissue. Alternatively, the present disclosure comprises at least calcitriol for use in a method of treating osteoarthritis or other condition characterised by degeneration of cartilage tissue. More preferably, the present disclosure comprises at least a retinoic acid or derivative thereof and calcitriol for use in a method of treating osteoarthritis or other condition characterised by degeneration of cartilage tissue.

In further preferred examples the present disclosure provides [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)carboxamido]benzoic acid (AM580), beta-estradiol, calcitriol, ciglitazone, kartogenin, lithium chloride, melatonin, and rhosin hydrochloride for use in a method of treating osteoarthritis or other condition characterised by degeneration of cartilage tissue. In addition, the present disclosure provides [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl) carboxamido] benzoic acid (AM580), calcitriol, leucovorin, forskolin and vorinostat for use in a method of treating osteoarthritis or other condition characterised by degeneration of cartilage tissue. Further still, the present disclosure provides all-trans retinoic acid, 9-cis retinoic acid, calcitriol, leucovorin and vorinostat for use in a method of treating osteoarthritis or other condition characterised by degeneration of cartilage tissue.

The present disclosure also provides a method of treating osteoarthritis, or other condition characterised by degeneration of cartilage tissue, the method comprising administering to an individual in need thereof, a composition comprising one or more of [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl) carboxamido]benzoic acid (AM580), all-trans retinoic acid, 9-cis retinoic acid, beta-estradiol, calcitriol, ciglitazone, kartogenin, lithium chloride, melatonin, rhosin hydrochloride, leucovorin, vorinostat and forskolin, thereby treating the osteoarthritis or other condition in the individual. Preferably, the composition comprises at least a retinoic acid or derivative thereof (such as [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl) carboxamido]benzoic acid (AM580), all-trans retinoic acid, 9-cis retinoic acid). Alternatively, the composition

comprises at least calcitriol. More preferably, the composition comprises at least a retinoic acid or derivative thereof and calcitriol.

Further, the present disclosure the use of one or more of [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl) carboxamido]benzoic acid (AM580), all-trans retinoic acid, 9-cis retinoic acid, beta-estradiol, calcitriol, ciglitazone, kartogenin, lithium chloride, melatonin, rhosin hydrochloride, leucovorin, vorinostat and forskolin, in the manufacture of a medicament for the treatment of osteoarthritis, or other condition characterised by degeneration of cartilage tissue. Preferably, the present disclosure comprises the use of at least a retinoic acid or derivative thereof (such as [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl) carboxamido]benzoic acid (AM580), all-trans retinoic acid, 9-cis retinoic acid), in the manufacture of a medicament for the treatment of osteoarthritis, or other condition characterised by degeneration of cartilage tissue. Alternatively, the present disclosure comprises the use of at least calcitriol in the manufacture of a medicament for the treatment of osteoarthritis, or other condition characterised by degeneration of cartilage tissue. More preferably, the present disclosure comprises the use of at least a retinoic acid or derivative thereof and calcitriol.

In further preferred examples the present disclosure provides the use of [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)carboxamido]benzoic acid (AM580), beta-estradiol, calcitriol, ciglitazone, kartogenin, lithium chloride, melatonin, and rhosin hydrochloride in the manufacture of a medicament for the treatment of osteoarthritis, or other condition characterised by degeneration of cartilage tissue. In addition, the present disclosure provides the use of [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl) carboxamido] benzoic acid (AM580), calcitriol, leucovorin, forskolin and vorinostat in the manufacture of a medicament for the treatment of osteoarthritis, or other condition characterised by degeneration of cartilage tissue. Further still, the present disclosure provides the use of all-trans retinoic acid, 9-cis retinoic acid, calcitriol, leucovorin and vorinostat in the manufacture of a medicament for the treatment of osteoarthritis, or other condition characterised by degeneration of cartilage tissue.

Still further, the present disclosure provides a pharmaceutical composition comprising one or more of [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)

carboxamido]benzoic acid (AM580), all-trans retinoic acid, 9-cis retinoic acid, beta-estradiol, calcitriol, ciglitazone, kartogenin, lithium chloride, melatonin, rhosin hydrochloride, leucovorin, vorinostat and forskolin, for use in a method of treating osteoarthritis or other condition characterised by degeneration of cartilage tissue. Preferably, the pharmaceutical composition comprises at least a retinoic acid or derivative thereof (such as [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl) carboxamido]benzoic acid (AM580), all-trans retinoic acid, 9-cis retinoic acid). Alternatively, the pharmaceutical composition comprises at least calcitriol. More preferably, the pharmaceutical composition comprises at least a retinoic acid or derivative thereof and calcitriol.

The present disclosure provides a pharmaceutical composition comprising [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)carboxamido]benzoic acid (AM580), beta-estradiol, calcitriol, ciglitazone, kartogenin, lithium chloride, melatonin, and rhosin hydrochloride for use in a method of treating osteoarthritis or other condition characterised by degeneration of cartilage tissue. In addition, the present disclosure provides a pharmaceutical composition comprising [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl) carboxamido] benzoic acid (AM580), calcitriol, leucovorin, forskolin and vorinostat for use in a method of treating osteoarthritis or other condition characterised by degeneration of cartilage tissue. Further still, the present disclosure provides a pharmaceutical composition comprising all-trans retinoic acid, 9-cis retinoic acid, calcitriol, leucovorin and vorinostat for use in a method of treating osteoarthritis or other condition characterised by degeneration of cartilage tissue.

Further examples of the present disclosure described in the preceding paragraphs will become apparent from the following description, given by way of example and with reference to the accompanying drawings.

It will be understood that the present disclosure disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative examples of the present disclosure.

CLAIMS

1. A method for reprogramming a source cell to produce a cell that exhibits at least one characteristic of a chondrocyte comprising:

i) providing a source cell, or a cell population comprising a source cell;

ii) contacting said source cell with one or more agents that activate or increase the expression of two or more transcription factors; and

iii) culturing said cell or cell population, and optionally monitoring the cell or cell population for at least one characteristic of a chondrocyte cell, wherein:

- the transcription factors are two or more of those listed in Tables 1 to 4.

2. A method according to claim 1 wherein the source cell is a pluripotent, multipotent or somatic cell.

3. A method according to claim 2 wherein the pluripotent cell is an embryonic stem cell.

4. A method according to claim 2 wherein the source cell is a fibroblast, dermal fibroblasts or de-differentiated chondrocyte.

5. The method according to claim 1 wherein the source cell is the dermal fibroblast and the transcription factors are any two or more of the transcription factors listed in Tables 1 or 4.

6. The method according to claim 1 wherein the source cell is the de-differentiated chondrocyte and the transcription factors are any two or more of the transcription factors listed in Tables 3.

7. A method of generating a cell exhibiting at least one characteristic of a chondrocyte from a de-differentiated chondrocyte, the method comprising:

- increasing the amount of two or more transcription factors, or variants thereof, in a de-differentiated chondrocyte; and

- culturing the de-differentiated chondrocyte for a sufficient time and under conditions to allow re-differentiation to a chondrocyte, wherein:

- the transcription factors are two or more of those listed in Table 3.

8. The method of claim 7, wherein the protein expression or amount of the transcription factors as shown in a single row of Table 3 is increased.

9. A method of preventing the de-differentiation of a chondrocyte cell culture, the method comprising:

- providing a de-differentiated chondrocyte cell, or a cell population comprising a de-differentiated chondrocyte cell;

- contacting said de-differentiated chondrocyte cell with one or more agents that activate or increase the expression of two or more transcription factors; and

- culturing said de-differentiated cell or cell population, and optionally monitoring the cell or cell population for at least one characteristic of a chondrocyte cell thereby preventing the de-differentiation of the chondrocyte and maintaining at least one characteristic of a chondrocyte in the chondrocyte cell culture,

- wherein the transcription factors are two or more of those listed in Table 3.

10. The method of any one of claims 1 to 9, wherein the protein expression or amount of the following transcription factors is increased:

a) IL11, SOX5, VEGFA, RUNX1, VDR, NR2F1, FOSB and PRRX1;

b) SOX9, TCF4, VEGFA, RUNX1, VDR, NR2F1, FOSB and PRRX1;

c) SOX9, SOX5, HOXA7, RUNX1, VDR, NR2F1, FOSB and PRRX1;

d) SOX9, SOX5, VEGFA, TCF4, VDR, NR2F1; FOSB and PRRX1;

e) SOX9, SOX5, VEGFA, RUNX1, TCF4, NR2F1, FOSB and PRRX1;

f) SOX9, SOX5, VEGFA, RUNX1, VDR, HOXA7, FOSB and PRRX1;

g) SOX9, SOX5, VEGFA, RUNX1, VDR, NR2F2, FOSB and PRRX1;

h) SOX9, SOX5, VEGFA, RUNX1, VDR, NR2F1, HOXA7 and PRRX1; or

i) SOX9, SOX5, VEGFA, RUNX1, VDR, NR2F1, FOSB and HOXA7;

11. The method of any one of claims 4 or 6 or 7 to 10, wherein the de-differentiated chondrocyte is obtained from an expanded culture of chondrocytes, or is obtained from a biological sample from an individual suffering from osteoarthritis.

12. The method of any one of claims 1 to 11, wherein the transcription factors are two or more of SOX9, SOX5, VEGFA, RUNX1, VDR, NR2F1, FOSB and PRRX1.

13. The method of any one of claims 1 to 11, wherein the transcription factors are SOX9, SOX5, VEGFA, RUNX1, VDR, NR2F1, FOSB and PRRX1.

14. The method of any one of the preceding claims wherein protein expression or the amount of the two or more transcription factors, or variants thereof, is increased in a source cell by contacting the source cell with an agent that increases the expression or amount of the two or more transcription factors, preferably wherein the agent is selected from the group consisting of: a nucleic acid, a protein, an aptamer, a small molecule, ribosome, an RNAi agent and peptide-nucleic acid (PNA), and analogues or variants thereof, and more preferably wherein the agent is a nucleic acid encoding one or more transcription factors listed in any one of Tables 1 to 4.

15. The method of any one of claims 1 to 14, wherein the protein expression or amount of the two or more transcription factors is increased in a source cell by contacting the source cell with one or more small molecules that increase the expression or amount of the two or more transcription factors, preferably wherein the one or more small molecules is selected from the group consisting of: [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2- naphthalenyl)carboxamido]benzoic acid (AM580), all-trans retinoic acid, 9-cis retinoic acid, beta-estradiol, calcitriol, ciglitazone, kartogenin, lithium chloride, melatonin, rhosin hydrochloride, leucovorin, vorinostat and forskolin.

16. The method of claim 15 wherein the source cell is contacted with [4-[(5,6,7,8- Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)carboxamido]benzoic acid (AM580), beta-estradiol, calcitriol, ciglitazone, kartogenin, lithium chloride, melatonin, and rhosin hydrochloride.

17. The method of claim 15, wherein the source cell is contacted with [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)carboxamido]benzoic acid (AM580), calcitriol, leucovorin, vorinostat and forskolin.

18. The method of claim 15, wherein the source cell is contacted with all-trans retinoic acid, 9-cis retinoic acid, calcitriol, leucovorin and vorinostat.

19. A method according to any one of claims 1 to 8, wherein culturing the source cell for a sufficient time and under conditions to allow differentiation to a chondrocyte includes culturing the cells for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 days in a relevant medium as shown in Table 6, preferably wherein the culturing of the cells is performed under hypoxic conditions.

20. A method according to any one of the preceding claims, wherein the cell exhibiting at least one characteristic of a chondrocyte is to be administered to an individual.

21. A cell exhibiting at least one characteristic of a chondrocyte, wherein the cell is produced by a method according to any one of claims 1 to 20.

22. A population of cells, wherein at least 5% of cells exhibit at least one characteristic of a chondrocyte, wherein the cells are produced by a method according to any one of claims 1 to 20.

23. The method of any one of claims 1 to 22, wherein the at least one characteristic of a chondrocyte is selected from a morphological marker, the expression of one or more genes or the production of one or more peptides or protein/polysaccharide complexes.

24. A population of cells according to claim 22, for use in treating osteoarthritis or other condition characterised by degeneration of cartilage tissue.

25. One or more of [4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2- naphthalenyl)carboxamido]benzoic acid (AM580), all-trans retinoic acid, 9-cis retinoic acid, beta-estradiol, calcitriol, ciglitazone, kartogenin, lithium chloride, melatonin, rhosin hydrochloride, leucovorin, vorinostat and forskolin, for use in treating osteoarthritis or other condition characterised by degeneration of cartilage tissue.

26. A composition comprising at least one chondrocyte and at least one agent which increases the activity or the protein expression of two or more transcription factors in the chondrocyte, wherein the transcription factor may be any of the transcription factors listed in Tables 1 to 4.