

CLAIMS

[Claim 1] A population of isolated mitochondria, wherein:

- (i) at least 80% of the mitochondria in the population have intact inner and outer membranes,
- (ii) at least 80% of the mitochondria in the population are polarized as measured by a fluorescence indicator,
- and/or
- (iii) at least 80% of the mitochondria in the population maintain functional capability in an extracellular environment.

[Claim 2] The population of isolated mitochondria of claim 1, wherein the functional capability in an extracellular environment of (iii) is measured by a fluorescence indicator of membrane potential.

[Claim 3] The population of isolated mitochondria of claim 1, wherein the extracellular environment of (iii) comprises a total calcium concentration of about 8 to about 12 mg/dL.

[Claim 4] The population of isolated mitochondria of claim 1, wherein the extracellular environment of (iii) comprises a free/active calcium concentration of about 4 to about 6 mg/dL.

[Claim 5] The population of isolated mitochondria of claim 1, wherein at least 80% of the mitochondria in the population are not undergoing dynamin-related protein 1 (drp1)-dependent division.

[Claim 6] The population of isolated mitochondria of claim 1, wherein the inner membranes of the mitochondria comprise densely folded cristae.

[Claim 7] The population of isolated mitochondria of any one of claims 1-6, wherein at least 80% of the mitochondria in the population have a non-filamentous shape.

[Claim 8] The population of isolated mitochondria of claim 7, wherein at least 85% of the mitochondria have a non-filamentous shape.

[Claim 9] The population of isolated mitochondria of claim 8, wherein at least 90% of the mitochondria have a non-filamentous shape.

[Claim 10] The population of isolated mitochondria of any one of claims 1-9, wherein the mitochondria exhibit decreased association with mitochondria-associated membrane (MAM) as measured by glucose regulated protein 75 (GRP75) expression.

[Claim 11] The population of isolated mitochondria of claim 10, wherein the decreased association is a decrease of at least about 30% relative to the association with MAM of mitochondria in a cell or of isolated mitochondria obtained by a method comprising homogenization of cells.

[Claim 12] The population of isolated mitochondria of claim 11, wherein the decreased association is a decrease of at least about 50%.

[Claim 13] The population of isolated mitochondria of any one of claims 1-12, wherein

- (i) at least 85% of the mitochondria in the population have intact inner and outer membranes,
- (ii) at least 85% of the mitochondria in the population are polarized as measured by a fluorescence indicator,
- and/or
- (iii) at least 85% of the mitochondria in the population maintain functional capability in an extracellular environment.

[Claim 14] The population of isolated mitochondria of any one of claims 1-12, wherein

- (i) at least 90% of the mitochondria in the population have intact inner and outer membranes,
- (ii) at least 90% of the mitochondria in the population are polarized as measured by a fluorescence indicator,
- and/or

(iii) at least 90% of the mitochondria in the population maintain functional capability in an extracellular environment.

[Claim 15] The population of isolated mitochondria of any one of claims 1-14, wherein the fluorescence indicator is selected from the group consisting of JC-1, tetramethylrhodamine methyl ester (TMRM), and tetramethylrhodamine ethyl ester (TMRE).

[Claim 16] The population of isolated mitochondria of any one of claims 1-15, wherein at least 80% of the mitochondria in the population are between about 500 nm and about 3500 nm in size.

[Claim 17] The population of isolated mitochondria of any one of claims 1-16, wherein the polydispersity index (PDI) of the population is about 0.2 to about 0.8,

[Claim 18] The population of isolated mitochondria of any one of claims 1-16, wherein the polydispersity index (PDI) of the population is about 0.2 to about 0.3.

[Claim 19] The population of isolated mitochondria of any one of claims 1-18, wherein the zeta potential of the population of mitochondria is between about -15 mV and about -40 mV.

[Claim 20] The population of isolated mitochondria of any one of claims 1-19, wherein upon contact of the population of isolated mitochondria with a population of cells, the isolated mitochondria are capable co-localization with endogenous mitochondria in the cells.

[Claim 21] The population of isolated mitochondria of any one of claims 1-19, wherein upon contact of the population of isolated mitochondria with a population of cells, the mitochondria are capable of fusing with endogenous mitochondria in the cells.

[Claim 22] The population of isolated mitochondria of claim 20, wherein the mitochondria are capable of co-localization with the endogenous mitochondria after storage at 4°C for at least 12 hours.

[Claim 23] The population of isolated mitochondria of claim 21, wherein the mitochondria are capable of fusing with the endogenous mitochondria after storage at 4°C for at least 12 hours.

[Claim 24] The population of isolated mitochondria of any one of claims 1-23, wherein at least 70% of the isolated mitochondria in the population are polarized as measured by a fluorescence indicator, after the population undergoes one or more freeze-thaw cycle.

[Claim 25] The population of isolated mitochondria of any one of claims 1-24, wherein the mitochondria are capable of co-localization with endogenous mitochondria, after the population undergoes one or more freeze-thaw cycle:

[Claim 26] The population of isolated mitochondria of claim 24 or 25, wherein the population is frozen at -80°C or colder for at least two weeks and then thawed at 20°C or colder within about 5 minutes.

[Claim 27] The population of isolated mitochondria of claim 26, wherein the population is thawed within about 1 minute.

[Claim 28] The population of isolated mitochondria of claim 26 or 27, wherein the population is frozen in liquid nitrogen for the at least two weeks.

[Claim 29] The population of isolated mitochondria of claim 28, wherein the population is frozen in liquid nitrogen for at least two months.

[Claim 30] The population of isolated mitochondria of any one of claims 24 to 29, wherein upon contact of the thawed population of mitochondria with a population of cells, the isolated mitochondria in the population are capable of fusion with endogenous mitochondria in the cells.

[Claim 31] A composition comprising the population of isolated mitochondria of any one of claims 1-30.

[Claim 32] A formulation comprising the composition of claim 31 and a pharmaceutically acceptable carrier.

[Claim 33] A method for isolating mitochondria from cells, the method comprising:

- (i) treating cells in a first solution with a surfactant at a concentration below the critical micellar concentration for the surfactant,
- (ii) removing the surfactant to form a second solution,
- (iii) incubating the cells in the second solution, and
- (iv) recovering mitochondria from the second solution.

[Claim 34] The method of claim 33, wherein the concentration of the surfactant in the first solution is about 50% or less of the critical micelle concentration for the surfactant.

[Claim 35] The method of claim 33 or 34, wherein the concentration of the surfactant in the first solution is about 10% or less of the critical micelle concentration for the surfactant.

[Claim 36] The method of any one of claims 33-35, wherein the surfactant is a nonionic surfactant.

[Claim 37] The method of any one of claims 33-36, wherein the surfactant is selected from the group consisting of Triton-X 100, Triton-X 114, Nonidet P-40, n-Dodecyl-D-maltoside, Tween-20, Tween-80, saponin and digitonin.

[Claim 38] The method of claim 37, wherein the surfactant is saponin or digitonin, and wherein the concentration of the surfactant in the first solution is less than about 400 μM .

[Claim 39] The method of claim 37, wherein the surfactant is saponin or digitonin, and wherein the concentration of the surfactant in the first solution is less than about 50 μM .

[Claim 40] The method of claim 37, wherein the surfactant is saponin or digitonin, and wherein the concentration of saponin or digitonin in the first solution is about 30 μM to about 40 μM .

[Claim 41] The method of any one of claims 33-40, wherein the first solution further comprises a buffer comprising one or more of a tonicity agent, osmotic modifier, or chelating agent.

[Claim 42] The method of claim 41, wherein the first solution comprises a tris buffer, sucrose, and a chelator.

[Claim 43] The method of any one of claims 33-42, wherein treating the cells in the first solution comprise incubating the cells in the first solution for about 2 minutes to about 30 minutes at room temperature.

[Claim 44] The method of any one of claims 33-43, wherein removing the surfactant comprises decreasing the surfactant in the solution to less than 10% of the surfactant concentration in the first solution.

[Claim 45] The method of any one of claims 33-44, wherein removing the surfactant comprises decreasing the surfactant in the solution to less than 1% of the surfactant concentration in the first solution.

[Claim 46] The method of any one of claims 33-45, wherein removing the surfactant comprises washing the cells with a buffer.

[Claim 47] The method of any one of claims 33-46, wherein incubating the second solution comprises incubating the cells in the second solution for about 5 minutes to about 30 minutes at about 4°C.

[Claim 48] The method of any one of claims 33-47, wherein recovering the mitochondria from the second solution comprises collecting the supernatant to recover the isolated mitochondria.

[Claim 49] The method of any one of claims 33-48, wherein recovering the mitochondria from the second solution comprises centrifuging the second solution and collecting the supernatant following centrifugation to recover the isolated mitochondria.

[Claim 50] The method of any one of claims 33-49, wherein the method further comprises freezing the isolated mitochondria.

[Claim 51] The method of claim 50, wherein the method comprises freezing the isolated mitochondria in a buffer comprising a cryoprotectant.

[Claim 52] A population of isolated mitochondria obtained by the method according to any one of claims 33-51.

[Claim 53] A method for treating a disease or disorder, the method comprising contacting cells of a subject in need thereof with a population of isolated mitochondria according to any one of claims 1-30 or a composition of claim 31 or a formulation of claim 32, wherein the disease or disorder is selected from the group consisting of diabetes (Type I and Type II), metabolic disease, ocular disorders associated with mitochondrial dysfunction, hearing loss, mitochondrial toxicity associated with therapeutic agents, cardiotoxicity associated with chemotherapy or other therapeutic agents, a mitochondrial dysfunction disorder, and migraine.

[Claim 54] A method for treating a disease or disorder associated with mitochondrial dysfunction, the method comprising contacting cells of a subject in need thereof with a population of isolated mitochondria according to any one of claims 1-30 or a composition of claim 31 or a formulation of claim 32.

[Claim 55] The method of claim 54, wherein the disease or disorder is selected from the group consisting of mitochondrial myopathy, diabetes and deafness (DAD) syndrome, Barth Syndrome, Leber's hereditary optic neuropathy (LHON), Leigh syndrome, NARP (neuropathy, ataxia, retinitis pigmentosa and ptosis syndrome), myoneurogenic gastrointestinal encephalopathy (MNGIE), MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes) syndrome, myoclonic epilepsy with ragged red fibers (MERRF) syndrome, Kearns-Sayre syndrome, and mitochondrial DNA depletion syndrome.

[Claim 56] The method of claim 54, wherein the disease or disorder is an ischemia-related disease or disorder.

[Claim 57] The method of claim 56, wherein the ischemia-related disease or disorder is selected from the group consisting of cerebral ischemic reperfusion, hypoxia ischemic encephalopathy, acute coronary syndrome, a myocardial infarction, a liver ischemia-reperfusion injury, an ischemic injury-compartmental syndrome, a blood vessel blockage, wound healing, spinal cord injury, sickle cell disease, and reperfusion injury of a transplanted organ.

[Claim 58] The method of claim 54, wherein the disease or disorder is a genetic disorder.

[Claim 59] The method of claim 54, wherein the disease or disorder is an aging disease or disorder.

[Claim 60] The method of claim 54, wherein the disease or disorder is a neurodegenerative condition or cardiovascular condition.

[Claim 61] The method of claim 60, wherein the neurodegenerative condition is selected from the group consisting of dementia, Friedrich's ataxia, amyotrophic lateral sclerosis, mitochondrial myopathy, encephalopathy, lactacidosis, stroke (MELAS), myoclonic epilepsy with ragged red fibers (MERFF), epilepsy, Parkinson's disease, Alzheimer's disease, or Huntington's Disease. Exemplary neuropsychiatric disorders include bipolar disorder, schizophrenia, depression, addiction disorders, anxiety disorders, attention deficit disorders, personality disorders, autism, and Asperger's disease.

[Claim 62] The method of claim 60, wherein the cardiovascular condition is selected from the group consisting of coronary heart disease, myocardial infarction, atherosclerosis, high blood pressure, cardiac arrest, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, congestive heart failure, arrhythmia, stroke, deep vein thrombosis, and pulmonary embolism.

[Claim 63] The method of claim 54, wherein the disease or disorder is a cancer, autoimmune disease, inflammatory disease, or fibrotic disorder.

[Claim 64] The method of claim 54, wherein the disease is acute respiratory distress syndrome (ARDS).

[Claim 65] The method of claim 54, wherein the disease or disorder is pre-eclampsia or intrauterine growth restriction (IUGR).

[Claim 66] The method of any one of claims 54-65, wherein the method comprises administering the population of isolated mitochondria or the composition to the subject via an intravenous, intra-arterial, intra-tracheal, subcutaneous, intramuscular, inhalation, or intrapulmonary route of administration.

[Claim 67] An isolated mitochondrion having intact inner and outer membranes, wherein the inner membrane comprises folded cristae, wherein the mitochondrion has been isolated from a cell, wherein the mitochondrion is polarized as measured by a fluorescence indicator, and wherein the mitochondrion is capable of maintaining polarization in an extracellular environment.

[Claim 68] The isolated mitochondrion of claim 67, wherein the mitochondrion has a non-filamentous shape.

[Claim 69] The isolated mitochondrion of claim 67 or 68, wherein voltage dependent anion channels (VDAC) on the surface of the mitochondrion are associated with tubulin at the surface.

[Claim 70] The isolated mitochondrion of claim 67, wherein the tubulin is dimeric tubulin.

[Claim 71] The isolated mitochondrion of claim 70, wherein the tubulin is a heterodimer comprising α -tubulin and β -tubulin.

[Claim 72] The isolated mitochondrion of any one of claims 67-71, wherein the fluorescence indicator is selected from the group consisting of JC-1, tetramethylrhodamine methyl ester (TMRM), and tetramethylrhodamine ethyl ester (TMRE).

[Claim 73] The isolated mitochondrion of any one of claims 67-72, wherein the isolated mitochondrion exhibits decreased association with mitochondria-associated membrane (MAM) as measured by glucose regulated protein 75 (GRP75) expression.

[Claim 74] The isolated mitochondrion of claim 73, wherein the decreased association is a decrease of at least about 30% relative to the association with MAM of a mitochondrion in a cell or of an isolated mitochondrion obtained by a method comprising homogenization of cells.

[Claim 75] The isolated mitochondrion of claim 74, wherein the decreased association is a decrease of at least about 50%.

[Claim 76] The isolated mitochondrion of any one of claims 67-75, wherein the membrane potential of the isolated mitochondrion is between about -30 mV and about -220 mV.

[Claim 77] The isolated mitochondrion of any one of claims 67-76, wherein the isolated mitochondrion is not undergoing drp1 dependent division.

[Claim 78] The isolated mitochondrion of any one of claims 67-77, wherein the isolated mitochondrion is between about 500 nm and about 3500 nm in size.

[Claim 79] A composition comprising the isolated mitochondrion of any one of claims 67-78.