DESCRIPTION

TITLE OF INVENTION

METHOD OF OBTAINING MITOCHONDRIA FROM CELLS AND OBTAINED MITOCHONDRIA

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to Japan application number 2019-136283, filed on July 24, 2019, the entire contents of which are hereby incorporated by reference.

BACKGROUND

[0002] Mitochondria are a type of organelle that plays three key roles: 1) metabolism such as ATP synthesis, 2) intracellular signaling such as Ca2+ and reactive oxygen species, and 3) control of cell death such as apoptosis and necrosis. In this sense, mitochondria are strongly associated with disease and have been studied by many researchers from a health perspective.

[0003] For mitochondrial function, the folded inner membrane and the surrounding outer membrane, and the electron transport system located in the inner membrane play a crucial role. The inner membrane forms a highly folded structure called cristae, which is believed to hold the supercomplex of electron transport system in the cristae membrane and to keep the proton concentration high by trapping the pumped protons in the cristae space. The electrochemical proton gradient formed by the electron transport system enables the transport of anions as well as ATP synthesis and cation transport.

[0004] Decreased mitochondrial function can cause a variety of diseases. There are currently no methods known in the art for isolating mitochondria from cells in a manner that retains mitochondrial function and structural integrity. This disclosure addresses this and other needs.

~~BRIEF~~ SUMMARY

[0005] The present disclosure provides a population of isolated or obtained or processed mitochondria, wherein the mitochondria in the population exhibit superior functional capability. For example, ~~in an aspect,~~ the present disclosure provides a population of isolated mitochondria wherein at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% of the mitochondria

in the population have intact inner and outer membranes; and/or at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% of the mitochondria in the population are polarized as measured by a fluorescence indicator. In ~~embodiment~~examples, the fluorescence indicator is selected from the group consisting of positively charged dyes such as JC-1, tetramethylrhodamine methyl ester (TMRM), and tetramethylrhodamine ethyl ester (TMRE).

[0005a] In one aspect, the present disclosure provides 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.

[0006] In ~~embodiment~~examples, the present disclosure provides a population of isolated mitochondria, wherein at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% of the mitochondria in the population maintain functional capability (e.g., are polarized) in an extracellular environment. In ~~embodiment~~examples, the functional capability in an extracellular environment is measured by a fluorescence indicator of membrane potential. In ~~embodiment~~examples, the fluorescence indicator is selected from the group consisting of positively charged dyes such as JC-1, TMRM, and TMRE. In ~~embodiment~~examples, the extracellular environment may comprise a total calcium concentration of about 4 mg/dL to about 12 mg/dL, or about 1 mmol/L (1000 μM) to about 3 mmol/L (3000 μM). For example, in ~~embodiment~~examples, the extracellular environment comprises a concentration of total calcium of about 8 mg/dL to about 12 mg/dL, or about 2 mmol/L (2000 μM) to about 3 mmol/L (3000 μM). In ~~embodiment~~examples, the extracellular environment comprises a concentration of free or active calcium of about 4 mg/dL to about 6 mg/dL, or about 1 mmol/L (1000 μM) to about 1.5 mmol/L (1500 μM). In ~~embodiment~~examples, the population of mitochondria maintain functional capability in an environment having a higher calcium concentration compared to the calcium environment in a cell.

[0007] In ~~embodiment~~examples, provided herein is a population of isolated mitochondria wherein at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% of the mitochondria in the population are not undergoing dynamin-related protein 1 (drp1) – dependent division. In ~~embodiment~~examples, provided herein is a population of isolated mitochondria having inner and outer membranes, wherein the inner membranes of the mitochondria comprise densely folded cristae.

[0008] In ~~embodiment~~examples, provided herein is a population of isolated mitochondria wherein at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% of the mitochondria in the population have a substantially non-filamentous, non-branched structure or shape. For example, in ~~embodiment~~examples, the mitochondria provided herein appear as round, dot-like, globular,

irregularly shaped, and/or slightly elongated, or any mixture thereof, when viewed under a microscope. In ~~embodiment~~examples, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% of the mitochondria in the population have a longer diameter to shorter diameter ratio of no more than 4:1, no more than 3.5:1, or no more than 3:1. In ~~embodiment~~examples, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% of the isolated mitochondria in the population of mitochondria provided herein have a length shorter than the double or triple of the hydrodynamic diameter of the mitochondrion. In this manner, the isolated mitochondria provided herein have a markedly different shape (non-filamentous) when compared to the shape of most mitochondria (filamentous) that are within cells. Thus, in ~~embodiment~~examples, the population of mitochondria provided herein has a shape that is distinct from mitochondria that exist in a cell and have not been isolated, in that at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% of the mitochondria in the population are non-filamentous in shape. In ~~embodiment~~examples, the population of isolated mitochondria provided herein exhibit decreased association with mitochondria-associated membrane (MAM). In ~~embodiment~~examples, the association with MAM is measured by expression of glucose regulated protein 75 (GRP75). In ~~embodiment~~examples, the population of isolated mitochondria provided herein exhibit about 60%, at least about 65%, at least about 70%, about 60%, about 50%, about 40%, about 30%, or less association with MAM when compared to mitochondria in a cell, and/or mitochondria that have been obtained by a conventional method of isolation such as one involving homogenization and/or high levels of detergent, as further described herein. In ~~embodiment~~examples, the population of isolated mitochondria provided herein exhibit a decrease in association with MAM, wherein the decrease is at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, or more relative to the association with MAM of mitochondria in a cell or of mitochondria isolated by a conventional method of isolation.

[0009] In ~~embodiment~~examples, the population of isolated mitochondria provided herein are between about 500 nm and about 3500 nm in size. In ~~embodiment~~examples, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% of the mitochondria in the population are between about 500 nm and about 3500 nm in size. In ~~embodiment~~examples, the average size of the mitochondria in the population is about 500 nm, about 600 nm, about 700 nm, about 800 nm,

about 900 nm, about 1000 nm, about 1100 nm, about 1200 nm, about 1300 nm, about 1400 nm, about 1500 nm, about 1600 nm, about 1700 nm, about 1800 nm, about 1900 nm, about 2000 nm, about 2100 nm, about 2200 nm, about 2300 nm, about 2400 nm, about 2500 nm, about 2600 nm, about 2700 nm, about 2800 nm, about 2900 nm, about 3000 nm, about 3100 nm, about 3200 nm, about 3300 nm, about 3400 nm, or about 3500 nm. In ~~embodiment~~examples, the polydispersity index (PDI) of the population of isolated mitochondria is about 0.2 to about 0.8. In ~~embodiment~~examples, the PDI of the population of isolated mitochondria is about 0.2 to about 0.5. In ~~embodiment~~examples, the PDI of the population of isolated mitochondria is about 0.25 to about 0.35. In ~~embodiment~~examples, the zeta potential of the population of mitochondria is about -15 mV to about -40 mV. In ~~embodiment~~examples, the zeta potential of the population of mitochondria is about -20 mV, about -25 mV, about -30 mV, about -35 mV, or about -40 mV.

[0010] In ~~embodiment~~examples, the population of isolated mitochondria provided herein are capable of being incorporated into cells and/or co-localization with endogenous mitochondria in cells, when the population of isolated mitochondria is contacted with a population of cells. For example, in ~~embodiment~~examples, the present disclosure provides methods for obtaining mitochondria from cells, and subsequently contacting a population of cells (*e.g.*, *ex vivo* or *in vivo* cells) with the population of isolated mitochondria. In such ~~embodiment~~examples, the mitochondria provided herein, which are isolated via the iMIT method described herein, are capable of co-localizing with the endogenous mitochondria present in the cells. In ~~embodiment~~examples, the mitochondria provided herein are further capable of fusing with the mitochondria present in the cells that they have contacted. In ~~embodiment~~examples, a substantial fraction of the population of isolated mitochondria are capable of co-localization and/or fusion with endogenous mitochondria in cells. For example, in ~~embodiment~~examples, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95% of the mitochondria in the population are capable of co-localization and/or fusion with endogenous mitochondria in cells. Thus, the mitochondria provided herein are markedly different from mitochondria isolated via conventional methods in that they are capable of co-localization and/or fusion with endogenous mitochondria in cells.

[0011] In ~~embodiment~~examples, the isolated mitochondria provided herein are stable and/or polarized and/or maintain membrane potential and/or maintain an intact inner and outer membrane and/or maintain the capacity to function after exposure to an extracellular environment (e.g., after exposure to a total calcium concentration of about 4 mg/dL to about 12 mg/dL), after storage at about 4°C. For example, in ~~embodiment~~examples, at least about 60%, at least about 65%, at least

about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% of the mitochondria in the population are stable and/or polarized and/or maintain membrane potential and/or maintain an intact inner and outer membrane and/or maintain the capacity to function after exposure to an extracellular environment (e.g., after exposure to a total calcium concentration of about 4 mg/dL to about 12 mg/dL), after storage at about 4°C. In ~~embodiment~~examples, the isolated mitochondria provided herein are stable and/or polarized and/or maintain membrane potential and/or maintain an intact inner and outer membrane and/or maintain the capacity to function after exposure to an extracellular environment (e.g., after exposure to a total calcium concentration of about 4 mg/dL to about 12 mg/dL), after storage at about -20°C or colder. For example, in ~~embodiment~~examples, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% of the mitochondria in the population are stable and/or polarized and/or maintain membrane potential and/or maintain an intact inner and outer membrane and/or maintain the capacity to function after exposure to an extracellular environment (e.g., after exposure to a total calcium concentration of about 4 mg/dL to about 12 mg/dL), after storage at about -20°C. In ~~embodiment~~examples, the isolated mitochondria provided herein are stable and/or polarized and/or maintain membrane potential and/or maintain an intact inner and outer membrane and/or maintain the capacity to function after exposure to an extracellular environment (e.g., after exposure to a total calcium concentration of about 4 mg/dL to about 12 mg/dL), after storage at about -80°C or colder. For example, in ~~embodiment~~examples, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% of the mitochondria in the population are stable and/or polarized and/or maintain membrane potential and/or maintain an intact inner and outer membrane and/or maintain the capacity to function after exposure to an extracellular environment (e.g., after exposure to a total calcium concentration of about 4 mg/dL to about 12 mg/dL), after storage at about -80°C. In ~~embodiment~~examples, the isolated mitochondria provided herein are stable and/or polarized and/or maintain membrane potential and/or maintain an intact inner and outer membrane and/or maintain the capacity to function after exposure to an extracellular environment (e.g., after exposure to a total calcium concentration of about 4 mg/dL to about 12 mg/dL), after storage in liquid nitrogen. For example, in ~~embodiment~~examples, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% of the mitochondria in the population are stable and/or polarized and/or maintain membrane

potential and/or maintain an intact inner and outer membrane and/or maintain the capacity to function after exposure to an extracellular environment (e.g., after exposure to a total calcium concentration of about 4 mg/dL to about 12 mg/dL), after storage in liquid nitrogen. In ~~embodiment~~examples, the storage is for at least about 2 hours, at least about 6 hours, at least about 12 hours, at least about 24 hours, at least about 48 hours, at least about 1 week, at least about 2 weeks, at least about 3 weeks, at least about 1 month, at least about 2 months, at least about 3 months, or longer. Thus, in ~~embodiment~~examples, the isolated mitochondria provided herein are markedly different from mitochondria isolated via conventional methods at least in that they maintain functional capacity when freshly isolated and even after storage.

[0012] In ~~embodiment~~examples, the isolated mitochondria provided herein are stable and/or polarized and/or maintain membrane potential and/or maintain an intact inner and outer membrane and/or maintain the capacity to function after exposure to an extracellular environment (e.g., after exposure to a total calcium concentration of about 4 mg/dL to about 12 mg/dL), after the population of mitochondria have been frozen for storage and then thawed.. In ~~embodiment~~examples, after being frozen and then thawed, the maintenance rate of the membrane potential is about 90% relative to the membrane potential of the mitochondria prior to freezing. For example, in ~~embodiment~~examples, the polarization ratio of a population of mitochondria that has been frozen and thawed is about 90% of the polarization ratio of that population prior to freezing. In ~~embodiment~~examples, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% of the mitochondria in the population are stable and/or polarized and/or maintain membrane potential and/or maintain an intact inner and outer membrane and/or maintain the capacity to function after exposure to an extracellular environment (e.g., after exposure to a total calcium concentration of about 4 mg/dL to about 12 mg/dL), after being frozen for storage and then thawed, for example, after being frozen for storage and then thawed one, two, three, or more times. Thus, in ~~embodiment~~examples, the isolated mitochondria provided herein are markedly different from mitochondria isolated via conventional methods at least in that they maintain functional capacity when even after being frozen for storage and then thawed.

[0013] In ~~embodiment~~examples, the population of isolated mitochondria provided herein are capable of being incorporated into cells and/or co-localization with and/or fusion with endogenous mitochondria in cells after storage of the mitochondria at any temperature provided herein (e.g., 4°C ± 3°C, -20°C ± 3°C, -80°C ± 3°C, or in liquid nitrogen). For example, in ~~embodiment~~examples, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%,

at least about 85%, at least about 90%, or at least about 95% of the mitochondria in the population capable of being incorporated into cells and/or co-localization with and/or fusion with endogenous mitochondria in cells after the mitochondria have been stored and/or undergone one or more freeze-thaw cycle. In ~~embodiment~~examples, the method of storing and thawing the population of isolated mitochondria provided herein comprises storing the population at about -20°C ± 3°C, about -80°C ± 3°C, or colder (e.g., in liquid nitrogen), and then thawing the mitochondria at about 20°C ± 3°C or colder, wherein the mitochondria are thawed within about 5 minutes, about 4 minutes, about 3 minutes, about 2 minutes, or about 1 minute. In particular ~~embodiment~~examples, the population of mitochondria is thawed within about 1 minute. Thus, in ~~embodiment~~examples, the mitochondria provided herein are markedly different from mitochondria isolated via conventional methods at least in that they are capable of being incorporated into cells and/or co-localization with and/or fusion with endogenous mitochondria in cells, whereas mitochondria isolated by conventional methods are incapable of or exhibit vastly reduced ability to being incorporated into cells and/or co-localize with and/or fuse with endogenous mitochondria in cells. In ~~embodiment~~examples, the co-localized isolated mitochondria can form a filamentous structure, a network structure, and/or a mesh-like structure.

[0014] In ~~embodiments~~another aspect, the present disclosure provides compositions comprising the isolated mitochondria provided herein. The compositions, in ~~embodiment~~examples, further comprise one or more pharmaceutically acceptable carrier.

[0014a] In another aspect, the present disclosure provides a formulation comprising the composition as disclosed herein and a pharmaceutically acceptable carrier.

[0015] In ~~embodiment~~examples, the present disclosure provides methods for isolating mitochondria from cells which differs from conventionally known methods and results in mitochondria having the superior functionality and other characteristics provided herein. In ~~embodiments~~ another aspect, the present disclosure provides the method for isolating mitochondria from cells comprises treating cells in a first solution with a surfactant at a concentration below the critical micelle concentration (CMC) for the surfactant, removing the surfactant to form a second solution, incubating the cells in the second solution, and recovering mitochondria from the second solution. In ~~embodiment~~examples the concentration of the surfactant in the first solution is about 50% or less of the CMC for the surfactant. For example, in ~~embodiment~~examples, the concentration of the surfactant in the first solution is about 40% or less, about 30% or less, about 20% or less, or about 10% or less of the CMC for the surfactant.

[0016] In ~~embodiment~~examples, the surfactant is a non-ionic surfactant. In ~~embodiment~~examples, 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. In ~~embodiment~~examples, the surfactant is saponin or digitonin. In ~~embodiment~~examples, the concentration of the surfactant is less than about 400

μM. For example, in ~~embodiment~~examples, the concentration of surfactant in the first solution is less than about 300 μM, less than about 200 μM, less than about 100 μM, or less than about 50 μM. In ~~embodiment~~examples, the concentration of the surfactant in the first solution is about 100 μM, about 75 μM, about 60 μM, about 50 μM, about 40 μM, about 30 μM, or about 20 μM. In ~~embodiment~~examples, the concentration of the surfactant in the first solution is about 20 μM to about 50 μM, or about 30 μM to about 40 μM.

[0017] In ~~embodiment~~examples, the first solution further comprises a buffer comprising one or more of a tonicity agent, osmotic modifier, or chelating agent. In ~~embodiment~~examples, the first solution comprises a tris buffer, sucrose, and a chelator.

[0018] In ~~embodiment~~examples, the step of treating the cells in the first solution comprising a low concentration of surfactant (e.g., below the CMC for the surfactant) comprises incubating the cells in the first solution for about 2 minutes to about 30 minutes at room temperature. For example, in ~~embodiment~~examples, the step of treating cells in the first solution comprises incubating the cells in the first solution for about 2, about 5, about 10, about 15, about 20, about 25, or about 30 minutes. The incubation may be carried out at a temperature of about 4°C to about 37°C.

[0019] In ~~embodiment~~examples, the step of removing the surfactant comprises decreasing the surfactant in the solution to less than 10% of the surfactant concentration in the first solution, or to less than 1% of the surfactant concentration in the first solution. In ~~embodiment~~examples, the step of removing the surfactant comprises washing the cells with a buffer.

[0020] In ~~embodiment~~examples, the step of incubating the second solution comprises incubating the cells in the second solution for about 5 minutes to about 30 minutes. For example, in ~~embodiment~~examples, the step of incubating the cells in the second solution comprises incubating the cells in the second solution for about 5, about 10, about 15, about 20, about 25, or about 30 minutes. In ~~embodiment~~examples, the step of incubating the cells in the second solution is carried out at a temperature of about 4°C ± 3°C or on ice.

[0021] In ~~embodiment~~examples, the step of recovering the mitochondria from the second solution comprises collecting the supernatant to recover the isolated mitochondria. In ~~embodiment~~examples, the step of recovering the mitochondria from the second solution comprises centrifuging the second solution and collecting the supernatant following centrifugation to recover the isolated mitochondria.

[0022] In ~~embodiment~~examples, the iMIT may be performed on a cell attaching to a culture surface. In ~~embodiment~~examples, the iMIT may be performed on a cell attaching to a culture surface without

detaching the cell from the surface. In ~~embodiment~~examples, the step of recovering the mitochondria from the second solution comprises collecting the supernatant to recover the isolated mitochondria, which can be optionally followed by washing the remaining cell on the culture surface with the second solution or another second solution to combine it with the supernatant.

[0023] In ~~embodiment~~examples, the methods provided herein further comprise freezing the isolated mitochondria. In ~~embodiment~~examples, the methods comprise freezing the mitochondria in a buffer comprising a cryoprotectant (e.g., glycerol). In ~~embodiment~~examples, the methods comprise freezing the mitochondria in the buffer in liquid nitrogen. In ~~embodiment~~examples, the methods further comprise thawing the mitochondria after freezing. In ~~embodiment~~examples, the methods for thawing the mitochondria comprise rapidly thawing the mitochondria, for example, within about 5 minutes or within about 1 minute. In ~~embodiment~~examples, the mitochondria are thawed in a warm bath having a temperature of about 20°C ± 3°C to about 37°C ± 3°C. In ~~embodiment~~examples, the mitochondria are thawed at a temperature of about 20°C ± 3°C or colder.

[0024] In ~~embodiments~~another aspect, the present disclosure provides a population of isolated mitochondria obtained by the method provided herein. In ~~embodiment~~examples, the method provided herein is the "iMIT" method and the mitochondria obtained by this method are referred to herein as "Q" mitochondria. In ~~embodiment~~examples, the present disclosure provides compositions and/or formulations comprising the population of isolated mitochondria obtained by the methods provided herein.

[0024a] In another aspect, the present disclosure provides use of a population of isolated mitochondria as disclosed herein or a composition as disclosed herein or a formulation as disclosed herein in the manufacture of a medicament for treating a disease or disorder, 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. In yet another aspect, the present disclosure provides use of a population of isolated mitochondria as disclosed herein or a composition as disclosed herein or a formulation as disclosed herein in the manufacture of a medicament for treating a disease or disorder associated with mitochondrial dysfunction.

[0025] In ~~embodiment~~examples, the present disclosure provides methods for treating or preventing a disease or disorder associated with mitochondrial dysfunction, the method comprising contacting cells of a subject with a population of isolated mitochondria provided herein, e.g., the Q mitochondria. In ~~embodiment~~examples, the disease or disorder is an ischemia-related disease or disorder. For example, in ~~embodiment~~examples, 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. In ~~embodiment~~examples, the disease or disorder is a genetic disorder. In ~~embodiment~~examples, the disease or disorder is a cancer, cardiovascular disease, ocular disorder, otic disorder, autoimmune disease, inflammatory disease, or fibrotic disorder. In ~~embodiment~~examples, the disease is acute respiratory distress syndrome (ARDS). In ~~embodiment~~examples, the disease or disorder is an aging disease or disorder, or a condition

associated with aging. In ~~embodiment~~examples, the disease or disorder is pre-eclampsia or intrauterine growth restriction (IUGR).

[0026] In ~~embodiment~~examples, the present disclosure provides methods for treating or preventing a disease or disorder provided herein, wherein the method comprises administering the population of isolated mitochondria or the composition to a subject in need thereof. In ~~embodiment~~examples, the route of administration of the isolated mitochondria is via an intravenous, intra-arterial, intra-tracheal, subcutaneous, intramuscular, inhalation, or intrapulmonary route of administration. In ~~embodiment~~examples, the subject is a mammal, e.g., a human.

[0027] In another aspect, the present disclosure provides 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. In ~~embodiment~~examples, the present disclosure provides 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 (e.g., JC-1, TMRM, or TMRE), and wherein the mitochondrion is capable of maintaining polarization in an extracellular environment. In ~~embodiment~~examples, the folded cristae are densely folded cristae. In ~~embodiment~~examples, the mitochondrion has a substantially non-filamentous shape. In ~~embodiment~~examples, the mitochondrion comprises voltage dependent anion channels (VDAC) on its surface that are associated with tubulin. For example, in ~~embodiment~~examples, the isolated mitochondrion comprises dimeric tubulin associated with VDAC on the surface. In ~~embodiment~~examples, the tubulin comprises at least α-tubulin. In ~~embodiment~~examples, the tubulin is a heterodimer comprising α-tubulin and β-tubulin. In ~~embodiment~~examples, the tubulin is a homodimer. In ~~embodiment~~examples, the isolated mitochondrion exhibits decreased association with MAM as measured by GRP75 expression. For example, in ~~embodiment~~examples, isolated mitochondrion exhibits about 70%, about 60%, about 50%, about 40%, about 30%, or less association with MAM when compared to mitochondrion that is present in a cell (i.e. has not been isolated), and/or a mitochondrion that has been obtained by a conventional method of isolation such as one involving homogenization and/or high levels of detergent, as further described herein. In ~~embodiment~~examples, the isolated mitochondrion provided herein exhibits a decrease in association with MAM, wherein the decrease is at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, or more relative to the association with MAM of a mitochondrion that is present in a cell (i.e., has not been isolated) and/a mitochondrion that has been isolated by a conventional method of isolation.

[0028] In ~~embodiment~~examples, the isolated mitochondrion provided herein has a membrane potential of between about -30 mV and about -220 mV. In ~~embodiment~~examples, the isolated mitochondrion is non-filamentous in shape. In ~~embodiment~~examples, the isolated mitochondrion is not undergoing drp1-

dpendent division. In ~~embodiment~~examples, the isolated mitochondrion is between about 500 nm and 3500 nm in size. For example, in ~~embodiment~~examples, the isolated mitochondrion is about 500, about 600, about 700, about 800 nm, about 900 nm, about 1000 nm, about 1100 nm, about 1200 nm, about 1500 nm, about 2000 nm, about 2500 nm, about 3000 nm, or about 3500 nm in size.

[0029] In ~~embodiment~~examples, the present disclosure provides an isolated mitochondrion obtained by the methods provided herein. In ~~embodiment~~examples, the present disclosure provides compositions and formulations comprising an isolated mitochondrion provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] FIG. 1A shows the distribution of the fluorescence intensity ratio (ratio of mitochondrial fluorescence intensity to background fluorescence intensity) of a fluorescent indicator to mitochondria depolarized with a depolarizing agent. In FIG. 1A, 97.5% of depolarized mitochondria exhibited fluorescence intensities of less than 1.2. Thus, in this experiment, mitochondria were considered to have a membrane potential when the fluorescence intensity ratio exceeded 1.2.

[0031] FIG. 1B shows a microscopic image of mitochondria obtained by the method of the present invention by transmitted light and a fluorescence image with a fluorescent indicator (TMRE) in the same region as the transmitted light image. Scale bars indicate 10 μm.

[0032] FIG. 2A shows a TMRE fluorescence image of mitochondria obtained by the DHF method compared to a TMRE fluorescence image of mitochondria obtained by a homogenization method. Scale bars indicate 25 μm.

[0033] FIG. 2B, top panel, shows the protein content (μg) of the mitochondrial fraction isolated by the DHF method vs. the homogenization method. FIG. 2B, bottom panel, shows the TMRE positive mitochondria (%) of the mitochondrial fraction isolated by the DHF method vs. the homogenization method. The DHF method resulted in a statistically significantly higher % TMRE positive fraction ($p<0.05$).

[0034] FIG. 2C shows a microscope image (top panels) and TMRE staining fluorescence image (bottom left panel) of isolated mitochondria. The images are merged in the bottom right panel, and shows that almost all mitochondria in the isolated population are polarized.

[0035] FIG. 2D shows an electron microscope image of a mitochondrion isolated by the methods provided herein. The scale bar is 1 μm. The image shows that the isolated mitochondrion has intact inner and outer membranes, and densely folded cristae.

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 if 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] Use of ~~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 in the manufacture of a medicament for treating a disease or disorder, 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] Use of ~~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 in the manufacture of a medicament for treating a disease or disorder associated with mitochondrial dysfunction.

[Claim 55] The ~~method~~ use 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~~ use of claim 54, wherein the disease or disorder is an ischemia-related disease or disorder.

[Claim 57] The ~~method~~ use 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~~ use of claim 54, wherein the disease or disorder is a genetic disorder.

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

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

[Claim 61] The ~~method~~ use 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~~ use 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~~ use of claim 54, wherein the disease or disorder is a cancer, autoimmune disease, inflammatory disease, or fibrotic disorder.

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

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

[Claim 66] The ~~method~~ use of any one of claims 54-65, wherein the ~~method~~ use comprises ~~administering~~ that the population of isolated mitochondria or the composition is to be administered 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.