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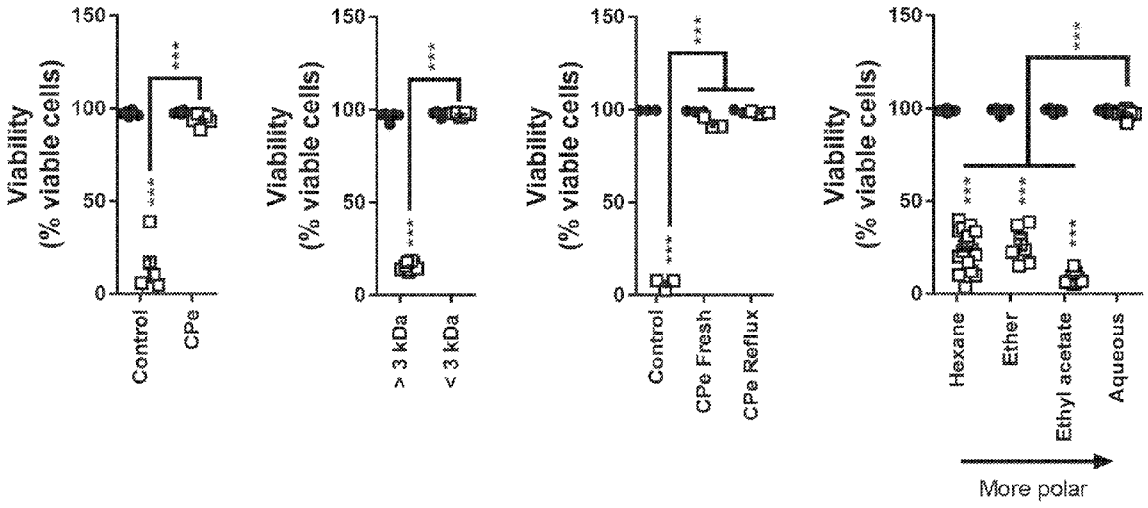

Fig. 4

(57) Abstract: Compositions and methods are disclosed for the identification of agents to treat neurological diseases and disorders, including neurodegenerative diseases. Screening assays assess protection of cultured human central nervous system (CNS) pericytes against stressors that contribute to the etiology of neurodegenerative and other CNS diseases by causing loss and/or dysfunction of such pericytes. Choroid plexus (CP) -derived compositions, including CP products previously thought to act directly on neuronal cells as neuroprotectants, are herein described to function as agents that protect human CNS pericytes against such stressors. CP-derived pericyte protective agents (PPA) thus may find uses in treating CNS and other diseases.

[Continued on next page]

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PERICYTE PROTECTIVE AGENTS FOR NEUROLOGICAL DISORDERS INCLUDING NEURODEGENERATIVE DISEASES, CENTRAL NERVOUS SYSTEM DISEASES AND OTHERS

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 62/580,942 filed November 2, 2017, which application is incorporated by reference herein in its entirety.

BACKGROUND

Technical Field

The present disclosure relates generally to neurological diseases and disorders, including neurodegenerative diseases, and to identification of agents for the treatment of such conditions. More specifically, compositions and methods are described pertaining to protection of central nervous system (CNS) pericytes and/or peripheral nervous system (PNS) pericytes from stressors that contribute to the etiology of disease by causing loss and/or dysfunction of such pericytes. As disclosed herein, pericyte protective agents (PPA) include choroid plexus (CP) –derived compositions that may be useful for treating neurological disorders including neurodegenerative disease, CNS disease, and other diseases and disorders.

Description of the Related Art

Diseases and disorders of the nervous system represent significant medical, social and economic challenges for which effective remedies remain elusive. Neurodegenerative diseases are often associated with aging and may be characterized by the progressive loss of neuronal cells from the central nervous system (CNS) and/or the peripheral nervous system

(PNS), often accompanied by depression or dementia and deterioration or loss of one or more of memory, motor skills, cognitive skills, and sensory abilities, along with other neurological deficits (Suksuphew et al., 2015 *World J. Stem Cells* 7:502; Schadt et al., 2014 *Front. Pharmacol.* 5:252). Alzheimer's disease, Parkinson's disease, Huntington's disease, schizophrenia, retinal degeneration (*e.g.*, retinal degenerative diseases including macular degeneration, diabetic retinopathy, retinitis pigmentosa), and other nervous system diseases and disorders have become societal burdens of growing prevalence and increasing impact on healthcare costs.

Disease-related degeneration of nervous system cells, which in healthy individuals are important contributors to normal nervous system maintenance and activity, can lead to compromised nervous system functions with deleterious consequences. For example, damage to or loss of nervous system cells that secrete significant bioactive molecules such as growth factors, differentiation factors, tissue repair factors, neurotransmitters, detoxifying proteins, protein chaperones or the like, can result in devastating diseases such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS) and other conditions. When the normal functions of the lost cells involve homeostatic maintenance of a physiological state or appropriate responses to changing physiological cues, therapeutic strategies that attempt simply to restore one or a small number of multiple depleted factors to patients in an unregulated manner are typically unsuccessful. Instead, an effective disease-modifying therapy should involve constantly readjusting the supply of all factors normally secreted by these cells at physiological concentrations and in a biologically responsive, regulated manner.

Recent alternative approaches for treating neurodegeneration therefore involve introducing into the CNS viable therapeutic cells that can restore, repair or functionally replace the damaged cells. By such approaches, it is believed that the replacement cells may respond flexibly and pleiotropically to environmental cues supplied by the local milieu, for instance, by progressing

through quantitative and/or qualitative changes in their gene expression and protein secretion profiles as CNS cell and tissue growth, differentiation, repair and/or remodeling may proceed. Such CNS cell replacement therapies have included attempts to replace cells that have been lost due to disease directly with primary effector cells, such as fetal midbrain tissue transplants that may differentiate into dopamine-producing neurons after being transplanted into patients with Parkinson's disease (Kordower et al., 1995 *N Engl J Med.* 332(17):1118; Lindvall, 1998 *Mov Disord.* 13 Suppl 1:83; Roitberg et al., 2004 *Neurol Res.* 26(4):355; Kefalopoulou et al., 2014 *JAMA Neurol.* 71(1):83; Bega et al., 2014 *JAMA* 311(6):617).

As a departure from direct CNS cell replacement therapy, other recently identified alternative therapies focus on supplying, to a damaged CNS tissue site, cells that are capable of ameliorating the CNS deficit indirectly. For example, choroid plexus (CP) cells have attracted particular attention in view of the recognized role of these specialized CNS cells in cerebrospinal fluid (CSF) production (*e.g.*, Damkier et al., 2013 *Physiol. Rev.* 93:1847). The choroid plexus (CP) is a specialized epithelial tissue within the ventricles of the brain. One of its key functions is to secrete cerebrospinal fluid (CSF) that provides neurotrophic, neuro-protective and neuro-restorative factors, as evidenced by behavioral improvement and histological data from various small animal and non-human primate disease model studies (Redzic et al., 2005 *Curr Top Dev Biol.* 71:1; Borlongan et al., 2004 *Stroke* 35(9):2206; Emerich et al., 2006 *Neurobiol Dis.* 23(2):471; Borlongan et al., 2008 *Cell Transplant* 16(10):987; Luo et al., 2013 *J Parkinsons Dis.* 3(3):275).

The function and turn-over rate of CSF in the CNS decreases significantly during aging, which may contribute to many neurodegenerative diseases that occur among the elderly (Redzic et al., 2005 *Curr Top Dev Biol.* 71:1; Chen et al., 2009 *Exp Gerontol.* 44(4):289; Chen et al., 2012 *Exp Gerontol.* 47(4):323). Recent findings indicate that CSF circulation through the interstitial space within the brain occurs more effectively during the sleep cycle

in animals (Xie et al., 2013 *Science* 342(6156):373), implying that reduced sleep can contribute to decreased clearance of waste byproducts and increased plaque formation, which are known to contribute to neurodegeneration (Iliff et al., 2014 *J Neurosci.* 34(49):16180). These findings, however, also imply that continuous production of CSF locally within a damaged site in the brain during the entire diurnal cycle may have clinically undesirable consequences. For example, excess CSF production at a damaged CNS site may result in dilution of active synaptic proteins or neurotransmitters to potentially suboptimal concentrations in the synaptic areas. It is therefore difficult to predict whether increasing CSF production would be a viable therapeutic strategy for the treatment of CNS disease.

Nevertheless, multiple efforts have been directed to the use of choroid plexus (CP) cell transplants by implantation in the CNS, by which CP-derived CSF components or other CP processes, products or metabolites may act as secondary effectors to restore damaged host tissues, most likely by reprogramming and/or restoration of various cell types in and around the implantation site. For practical and ethical reasons, CP implants have typically employed xenogeneic CP cells and therefore require the implementation of immunosuppressive, anti-inflammatory measures to counteract immunological rejection and/or host inflammation (*e.g.*, foreign body response) reactions to the xenotransplants.

Biocompatible, semi-permeable alginate capsules are known as non-immunogenic vehicles in which to introduce therapeutic cells into the brain to minimize such reactions whilst permitting soluble cell products to diffuse into the tissue surrounding the implanted capsule (*e.g.*, US6322804, US5834001, US6083523, US2007/134224, US5869463, US2004/213768, US2009/0047325). The specific implantation in the brain of choroid plexus tissue fragments within biocompatible capsules for the treatment of CNS diseases is described, for example, in US2007/134224, and in US2004/213768 and US2009/0047325 and related patent application publications.

As described, for example, in US2009/0047325, in addition to CSF production by encapsulated xenotransplanted CP cells locally at an implantation site of CNS tissue damage, the use of neonatal CP cells may provide higher concentrations of biologically active CSF molecules than would be supplied by adult CP cells, given that the CSF of newborn mammals is typically enriched in CSF components. More recently, implantation has been described of potent, encapsulated long-lived CP cells that are induced with a CP-inducing agent to produce one or more cerebrospinal fluid (CSF) components at an altered level relative to the level produced without the step of induction by a CP-inducing agent (*see, e.g.,* US 2016/0361365 and WO 2016/187067).

From the foregoing, it will be appreciated that therapeutic strategies aimed at restoring the CNS neuronal compartment have been the focus of efforts to address neurodegenerative disease. More recently, however, evidence has emerged that certain non-neuronal CNS components may play significant roles in the pathogenesis of neurodegenerative disease and therefore represent potential new candidate targets for therapeutic intervention.

Vasculature-associated pericytes have long been known as cells that surround capillary endothelia in substantially all mammalian tissues, including in the CNS and PNS. These cells are believed to play a role, for instance, in regulating capillary lumenal diameter. In the CNS, pericytes have been identified as being important to the maintenance and regulation of the blood-brain barrier (BBB), and pericyte-to-endothelial cell ratios are higher in the brain than in any other tissue. By phenotypic and functional criteria, human CNS pericytes differ from the pericytes found in other tissues. The roles of human CNS pericytes in immunoregulation and neuroinflammation are slowly being elucidated, as the abilities to culture these cells and elicit physiologically relevant functional behaviors have only recently been described (*e.g.*, Rustenhoven et al., 2015 *Sci. Rep.* 5:12132; Jansson et al., 2014 *J.*

Neuroinflamm. 11:104; Rustenhoven et al., 2016 *J. Neuroinflamm.* 13:1; Jansson et al., 2016 *J. Neuroinflamm.* 13:1; Rustenhoven et al., 2017 *Trends Pharmacol. Sci.* 38:291).

CNS pericyte roles have thus been described, for example, in forming and maintaining the BBB, regulating blood flow to the brain, non-glial scar formation, and neuroinflammation, but a direct human CNS pericyte role in response to neurotrophic, neuro-protective and/or neuro-restorative factors has not been heretofore demonstrated in the context of neurodegenerative disease.

For example, there are at least two ways that CNS pericytes are thought to play a role in the disease progression of Parkinson's disease (PD). First, pericytes can mediate the transfer of α-synuclein aggregates through intercellular tunnelling nanotubes (TNTs) (*e.g.,* Dieriks et al., 2017 *Sci. Rep.* 7:42984). α-Synuclein (α-syn) is the main protein component of Lewy bodies, and the presence of α-syn aggregates is a characteristic feature of PD. TNTs, on the other hand, play a general role in intercellular communication and under normal conditions allow the transfer of materials, such as proteins and RNA, between cells. TNT formation also seems particularly important during mitosis. Human brain pericytes use TNTs as a mechanism for α-syn transfer, although these cells frequently form TNTs that permit the intercellular transfer of mis-folded aggregates of α-syn. It is this mode of action by which pericytes participate in PD disease progression, and elucidation of a clear mechanism for this transfer may offer a therapeutic target for PD treatment.

Another role that CNS pericytes play in the disease progression of PD and other brain diseases is by mediating neuroinflammation. Pericytes drive the inflammatory response in the CNS through the recruitment of neuroinflammatory cells such as microglia and astrocytes. However, while neuroinflammation occurs as part of normal CNS function, it can also have a detrimental effect on brain function and is present in almost every neurological disorder. Loss of capillary pericyte coverage is also related to compromised blood brain barrier (BBB) function, resulting in leakage from the vasculature

(*e.g.*, of inflammatory cell infiltrates and/or inflammatory macromolecules that would otherwise be excluded) into the CNS as a further mechanism by which pericyte dysfunction promotes neuroinflammation.

Clearly, there remains an unmet need in the art for enhancing CNS pericyte health, promoting CNS pericyte viability, and/or for reducing CNS pericyte contribution to deleterious inflammatory processes. These and related interventions at the level of the CNS pericyte would, for example, help reduce detrimental TNT activities, repair BBB damage and/or otherwise provide an alternative to CNS neuronal cells as therapeutic targets for treating neurodegenerative disease. The presently disclosed embodiments address these needs and provide other related advantages.

BRIEF SUMMARY

The present invention provides, in certain embodiments, a method of for identifying a pericyte protective agent (PPA) that protects a central nervous system (CNS) pericyte from a pericyte stressor, wherein said pericyte stressor is capable of inducing at least one of CNS pericyte loss and CNS pericyte dysfunction, the method comprising (a) contacting, simultaneously or sequentially and in any order, (i) a cultured human CNS pericyte with (ii) the pericyte stressor that is capable of inducing at least one of CNS pericyte loss and CNS pericyte dysfunction, and (iii) a candidate pericyte protective agent (PPA), under conditions and for a time sufficient to induce detectable CNS pericyte loss or detectable CNS pericyte dysfunction when the PPA is absent, thereby to obtain a human CNS pericyte test culture; and (b) detecting, in the human CNS pericyte test culture of (a), a level of at least one of CNS pericyte loss and CNS pericyte dysfunction that is decreased relative to the level that is detected when the PPA is absent, and thereby identifying the candidate PPA as a pericyte protective agent (PPA).

In certain other embodiments there is provided a screening method for identifying a pericyte protective agent (PPA) that protects a peripheral nervous system (PNS) pericyte from a pericyte stressor, wherein said pericyte stressor is capable of inducing at least one of PNS pericyte loss and PNS pericyte dysfunction, the method comprising (a) contacting, simultaneously or sequentially and in any order, (i) a cultured human PNS pericyte with (ii) the pericyte stressor that is capable of inducing at least one of PNS pericyte loss and PNS pericyte dysfunction, and (iii) a candidate pericyte protective agent (PPA), under conditions and for a time sufficient to induce detectable PNS pericyte loss or detectable PNS pericyte dysfunction when the PPA is absent, thereby to obtain a human PNS pericyte test culture; and (b) detecting, in the human PNS pericyte test culture of (a), a level of at least one of PNS pericyte loss and PNS pericyte dysfunction that is decreased relative to the level that is detected when the PPA is absent, and thereby identifying the candidate PPA as a pericyte protective agent (PPA).

In certain further embodiments the candidate PPA is produced by mammalian choroid plexus (CP) cells, and in certain still further embodiments the candidate PPA is present in a culture medium that has been conditioned by the mammalian CP cells. In a still further embodiment the culture medium has been separated from the mammalian CP cells and has been conditioned by at least one of (i) mammalian CP cells that are present in one or more semi-permeable biocompatible capsules in which are encapsulated choroid plexus (CP) tissue fragments that are obtained by either or both of mechanical and enzymatic dissociation of mammalian choroid plexus tissue to obtain CP cell clusters that are about 50 μm to at least about 200 μm in diameter and that comprise CP epithelial cells; (ii) cultured non-encapsulated CP cells obtained from mammalian choroid plexus tissue; and (iii) choroid plexus (CP) cells that are obtained by culturing a population of mammalian pluripotent cells under conditions and for a time sufficient to obtain a plurality of *in vitro* differentiated choroid plexus (CP) cells.

In another related embodiment, the mammalian CP cells are present in one or more semi-permeable biocompatible capsules in which are encapsulated choroid plexus (CP) tissue fragments that are obtained by either or both of mechanical and enzymatic dissociation of mammalian choroid plexus tissue to obtain CP cell clusters that are about 50 µm to at least about 200 µm in diameter and that comprise CP epithelial cells. In a further embodiment substantially all of said capsules are about 400 µm to about 800 µm in diameter and have about 200 to about 10,000 CP cells per capsule.

In certain other embodiments of the above described methods, the mammalian CP cells are present in one or more semi-permeable biocompatible capsules in which are encapsulated *in vitro* differentiated choroid plexus (CP) cells that are obtained by culturing a population of mammalian pluripotent cells under conditions and for a time sufficient to obtain a plurality of *in vitro* differentiated choroid plexus (CP) cells, substantially all of said capsules being about 400 µm to about 800 µm in diameter and having about 200 to about 10,000 CP cells per capsule. In certain embodiments the mammalian choroid plexus cells are from mammalian choroid plexus tissue of a mammal that is allogeneic or xenogeneic relative to the cultured human CNS pericyte or PNS pericyte. In certain embodiments the mammalian choroid plexus tissue comprises human, porcine, ovine, bovine, caprine, or non-human primate choroid plexus cells. In certain embodiments the porcine choroid plexus cells are cultured from a tissue that comprises fetal or neonatal choroid plexus tissue.

In certain embodiments the mammalian choroid plexus tissue is substantially free of human pathogens, and in certain embodiments the choroid plexus tissue is substantially free of human-tropic transmissible porcine endogenous retroviruses. In certain embodiments at least one of: (i) the choroid plexus tissue is substantially incapable of producing infectious human-tropic porcine endogenous retroviruses (PERVs), or (ii) the choroid plexus tissue is obtained from an animal that lacks PERV genes. In certain embodiments the choroid plexus tissue is obtained from an animal that lacks a

PERV-C *env* gene which is capable of recombination with a PERV-A *env* gene. In certain further embodiments the animal that lacks a PERV-C *env* gene which is capable of recombination with a PERV-A *env* gene has been genetically engineered to lack any or all PERV genes, which in certain further embodiments is an animal that is produced by Clustered Regularly-Interspaced Short Palindromic Repeats (CRISPR)-Cas9 editing.

In certain embodiments of the above described methods either one or both of (i) the population of mammalian pluripotent cells is obtained from a source that is selected from embryonic cells, umbilical cord cells, placental cells, neural crest progenitors, adult tissue stem cells, and somatic tissue cells; and (ii) the population of mammalian pluripotent cells is cultured in a culture medium that comprises one or more *in vitro* CP differentiation agents selected from a bone morphogenic protein (BMP) or a BMP signaling pathway agonist, a transforming growth factor-beta (TGF-β) superfamily member or a TGF-β signaling pathway agonist, a nodal protein or a nodal signaling pathway agonist, a mammalian growth and differentiation factor (GDF) or a GDF signaling pathway agonist, a Wnt protein ligand or a Wnt signaling pathway agonist, a fibroblast growth factor (FGF) or an FGF signaling pathway agonist, and sonic hedgehog (Shh) or a Shh signaling pathway agonist, under conditions and for a time sufficient to obtain said plurality of *in vitro* differentiated choroid plexus (CP) cells. In further embodiments the Wnt signaling pathway agonist is selected from WAY-316606 (SFRP inhibitor), IQ1 (PP2A activator), QS11 (ARFGAP1 activator), 2-amino-4-[3,4-(methylenedioxy) benzyl-amino]-6-(3-methoxyphenyl) pyrimidine, Norrin, R-spondin-1, R-spondin-2, R-spondin-3, or R-spondin-4, lithium chloride, lithium carbonate, lithium citrate, lithium orotate, lithium bromide, lithium fluoride, lithium iodide, lithium acetate, lithium hydroxide, lithium aluminum hydride, lithium perchlorate, lithium nitrate, lithium diisopropylamide, lithium borohydride, lithium oxide, lithium sulfate, lithium hexafluorophosphate, lithium tetroxide, lithium sulfide, lithium hydride, lithium amide, lithium lactate, lithium tetrafluoroborate, lithium dimethylamide, lithium phosphate, lithium peroxide, lithium manganese oxide,

lithium methoxide, lithium metaborate, lithium stearate, or another lithium salt that comprises cationic lithium.

In certain embodiments of the methods described above, the pericyte stressor comprises one or more agents selected from hydrogen peroxide, nitric oxide, tert-butylhydroperoxide, heavily-oxidized glycated LDL, and a pro-apoptotic agent. In certain other embodiments of the methods described above, the level of pericyte loss comprises a level of one or more of pericyte cell death, pericyte apoptosis, pericyte necrosis, and pericyte autophagy. In certain other embodiments of the methods described above, the level of pericyte dysfunction comprises a level of one or more of reactive oxygen species (ROS) production, reactive nitrogen species (RNS) production, matrix metalloproteinase 2 (MMP2) production, matrix metalloproteinase 9 (MMP9) production, angiopoietin 1 production, fibronectin 1 production, platelet derived growth factor receptor beta (PDGFRβ) expression, connexin 43 expression, NG2 expression, and IL-17R(A/C) expression. In certain other embodiments of the methods described above, the candidate PPA is a cerebrospinal fluid (CSF) component that is produced by choroid plexus (CP) cells.

Turning to another embodiment, there is provided a method for treating, reducing severity of, or reducing likelihood of occurrence of a central nervous system (CNS) condition associated with CNS pericyte loss or CNS pericyte dysfunction in a subject, comprising administering to the subject a pericyte protective agent (PPA) produced by a choroid plexus (CP) composition, under conditions that permit the PPA to contact CNS pericytes in the subject to decrease a level of CNS pericyte loss or CNS pericyte dysfunction in the subject relative to the level of CNS pericyte loss or CNS pericyte dysfunction in the subject when the PPA is absent, and thereby treating, reducing the severity of, or reducing the likelihood of occurrence of the central nervous system (CNS) condition associated with CNS pericyte loss or CNS pericyte dysfunction. In a further embodiment the central nervous system (CNS) condition associated with CNS pericyte loss or CNS pericyte dysfunction is selected from (a) a neurodegenerative disease that is characterized by death

of neurons, and (b) a nervous system disease that is selected from Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS, also known as motor neurone disease), ataxia-telangiectasia, progressive bulbar palsy, progressive muscular atrophy, dementia with Lewy bodies, multiple system atrophy, spinocerebellar ataxia type 1 (SCA 1), a retinal degenerative disease, or an age-related neurodegenerative disorder.

In another further embodiment, the central nervous system (CNS) condition associated with CNS pericyte loss or CNS pericyte dysfunction is selected from (a) a disease that is characterized by a decrease in a level of at least one nerve cell function, relative to the level of said nerve cell function in a control subject known to be free of the nervous system disease, and (b) the disease of (a) that is selected from Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis, and depression.

In another further embodiment the central nervous system (CNS) condition associated with CNS pericyte loss or CNS pericyte dysfunction is selected from (a) a disease that is characterized by an increase in a level of at least one nerve cell function, relative to the level of said nerve cell function in a control subject known to be free of the nervous system disease, and (b) the disease of (a) that is selected from psychosis, schizophrenia, epileptic seizures, ischemic stroke, and insomnia associated with restless leg syndrome.

In another further embodiment the central nervous system (CNS) condition associated with CNS pericyte loss or CNS pericyte dysfunction is selected from (a) a disease that is characterized by presence in the subject of cerebrospinal fluid (CSF) that comprises an altered level of one or more cerebrospinal fluid (CSF) components, relative to the level of said CSF component or components in a control subject known to be free of the nervous system disease, and (b) the disease of (a) that is selected from Alzheimer's disease and diabetes mellitus. In another further embodiment the central nervous system (CNS) condition associated with CNS pericyte loss or CNS pericyte dysfunction is selected from (a) a disease that is characterized by presence in the subject of an altered level of at least one choroid plexus function, relative to the level of said choroid plexus function in a control subject

known to be free of the nervous system disease, (b) the disease of (a) that is selected from Sturge-Weber syndrome and Klippel-Trenaunay-Weber syndrome, (c) a disease that is characterized by an increase in a level of abnormally folded protein deposits in brain tissue of the subject, relative to the level of abnormally folded protein deposits in a control subject known to be free of the nervous system disease, and (d) the disease of (c) that is selected from cerebral amyloid angiopathy, hereditary cerebral hemorrhage with amyloidosis-Icelandic type (HCHWA-I), cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D), meningocerebrovascular and oculoleptomeningeal amyloidosis, gelsolin-related spinal and cerebral amyloid angiopathy, familial amyloidosis-Finnish type (FAF), vascular variant prion cerebral amyloidosis, familial British dementia (FBD) (also known as familial cerebral amyloid angiopathy-British type or cerebrovascular amyloidosis-British type), familial Danish dementia (also known as heredopathia ophthalmo-oto-encephalica), familial transthyretin (TTR) amyloidosis, and PrP cerebral amyloid angiopathy (PrP-CAA); and (e) a disease that is caused by blood brain barrier (BBB) dysfunction.

In another further embodiment, the central nervous system (CNS) condition associated with CNS pericyte loss or CNS pericyte dysfunction is at least one of (i) a neurodegenerative disease that is characterized by death of CNS neurons, and (ii) a CNS disease characterized by a decrease in a level of at least one CNS nerve cell function, relative to the level of said CNS nerve cell function in a control subject known to be free of the CNS disease, and iii) a CNS disease characterized by an increase in a level of at least one CNS nerve cell function, relative to the level of said CNS nerve cell function in a control subject known to be free of the CNS disease, wherein said CNS neurons and CNS nerve cell are present in at least one of brain, spinal cord, retina, optic nerve, cranial nerve, olfactory nerve or olfactory epithelium. In certain other embodiments the central nervous system (CNS) condition associated with CNS pericyte loss or CNS pericyte dysfunction is one of Parkinson's disease, Alzheimer's disease, and Huntington's disease. In certain embodiments the retinal degenerative disease is selected from macular degeneration, diabetic retinopathy, and retinitis pigmentosa.

In another embodiment of the presently disclosed invention, there is provided a method for treating, reducing severity of, or reducing likelihood of occurrence of a peripheral nervous system (PNS) condition associated with PNS pericyte loss or PNS pericyte dysfunction in a subject, comprising administering to the subject a pericyte protective agent (PPA) produced by a choroid plexus (CP) composition, under conditions that permit the PPA to contact PNS pericytes in the subject to decrease a level of PNS pericyte loss or PNS pericyte dysfunction in the subject relative to the level of PNS pericyte loss or PNS pericyte dysfunction in the subject when the PPA is absent, and thereby treating, reducing the severity of, or reducing the likelihood of occurrence of the peripheral nervous system (PNS) condition associated with PNS pericyte loss or PNS pericyte dysfunction. In certain embodiments the condition associated with PNS pericyte loss or PNS pericyte dysfunction is at least one of (i) a neurodegenerative disease that is characterized by death of PNS neurons, and (ii) a PNS disease characterized by a decrease in a level of at least one PNS nerve cell function, relative to the level of said PNS nerve cell function in a control subject known to be free of the PNS disease, and iii) a PNS disease characterized by an increase in a level of at least one PNS nerve cell function, relative to the level of said PNS nerve cell function in a control subject known to be free of the PNS disease, wherein said PNS neurons and PNS nerve cell are present in at least one of a peripheral ganglion or a peripheral nerve.

Turning to another herein disclosed embodiment, there is provided a screening method for identifying a pericyte protective agent (PPA) that protects a central nervous system (CNS) pericyte from a pericyte stressor, wherein said pericyte stressor is capable of inducing at least one of CNS pericyte loss and CNS pericyte dysfunction, the method comprising (a) contacting, simultaneously or sequentially and in any order, (i) a cultured human CNS pericyte, (ii) the pericyte stressor that is capable of inducing at least one of CNS pericyte loss and CNS pericyte dysfunction, (iii) a conditioned culture medium that has been conditioned by mammalian choroid plexus (CP) cells and that contains a pericyte protective agent (PPA) produced by said CP cells, or an isolated fraction of said conditioned culture medium that contains

the PPA, which PPA is capable of decreasing in the cultured human CNS pericyte a level of at least one of CNS pericyte loss and CNS pericyte dysfunction, relative to the level that is detected when the PPA is absent, and (iv) one or a plurality of antibodies that have been generated against a portion of the culture medium that has been conditioned by mammalian choroid plexus (CP) cells and which contains the pericyte protective agent (PPA) produced by said CP cells, under conditions and for a time sufficient to induce detectable CNS pericyte loss or detectable CNS pericyte dysfunction when the PPA is absent, thereby to obtain a human CNS pericyte test culture; (b) detecting, in the human CNS pericyte test culture of (a), a level of at least one of CNS pericyte loss and CNS pericyte dysfunction that is decreased to a lesser degree relative to the level that is detected when the one or plurality of antibodies is absent, thereby indicating that the one or plurality of antibodies is capable of neutralizing the PPA; and (c) isolating the PPA by binding to at least one of the antibodies that is capable of neutralizing the PPA.

In certain further embodiments, isolating the PPA comprises isolating an immune complex that comprises at least one of the antibodies that is capable of neutralizing the PPA and the PPA. Certain further embodiments further comprise separating the at least one antibody that is capable of neutralizing the PPA from the PPA and structurally characterizing the PPA. In certain embodiments the culture medium has been separated from the mammalian CP cells and has been conditioned by at least one of (i) mammalian CP cells that are present in one or more semi-permeable biocompatible capsules in which are encapsulated choroid plexus (CP) tissue fragments that are obtained by either or both of mechanical and enzymatic dissociation of mammalian choroid plexus tissue to obtain CP cell clusters that are about 50 μm to at least about 200 μm in diameter and that comprise CP epithelial cells; (ii) cultured non-encapsulated CP cells obtained from mammalian choroid plexus tissue; and (iii) choroid plexus (CP) cells that are obtained by culturing a population of mammalian pluripotent cells under conditions and for a time sufficient to obtain a plurality of *in vitro* differentiated choroid plexus (CP) cells.

In certain embodiments the mammalian CP cells are present in one or more semi-permeable biocompatible capsules in which are encapsulated choroid plexus (CP) tissue fragments that are obtained by either or both of mechanical and enzymatic dissociation of mammalian choroid plexus tissue to obtain CP cell clusters that are about 50 µm to at least about 200 µm in diameter and that comprise CP epithelial cells. In certain further embodiments substantially all of said capsules are about 400 µm to about 800 µm in diameter and have about 200 to about 10,000 CP cells per capsule. In certain embodiments the mammalian CP cells are present in one or more semi-permeable biocompatible capsules in which are encapsulated *in vitro* differentiated choroid plexus (CP) cells that are obtained by culturing a population of mammalian pluripotent cells under conditions and for a time sufficient to obtain a plurality of *in vitro* differentiated choroid plexus (CP) cells, substantially all of said capsules being about 400 µm to about 800 µm in diameter and having about 200 to about 10,000 CP cells per capsule. In certain embodiments the mammalian choroid plexus cells are from mammalian choroid plexus tissue of a mammal that is allogeneic or xenogeneic relative to the cultured human CNS pericyte. In certain further embodiments the mammalian choroid plexus tissue comprises human, porcine, ovine, bovine, caprine, or non-human primate choroid plexus cells. In certain further embodiments the porcine choroid plexus cells are cultured from a tissue that comprises fetal or neonatal choroid plexus tissue. In certain further embodiments the mammalian choroid plexus tissue is substantially free of human pathogens. In certain embodiments the choroid plexus tissue is substantially free of human-tropic transmissible porcine endogenous retroviruses. In certain embodiments at least one of: (i) the choroid plexus tissue is substantially incapable of producing infectious human-tropic porcine endogenous retroviruses (PERVs), or (ii) the choroid plexus tissue is obtained from an animal that lacks PERV genes. In certain embodiments the choroid plexus tissue is obtained from an animal that lacks a PERV-C *env* gene which is capable of recombination with a PERV-A *env* gene. In certain further embodiments the animal that lacks a PERV-C *env* gene which is capable of

recombination with a PERV-A *env* gene has been genetically engineered to lack any or all PERV genes. In certain further embodiments the animal that has been genetically engineered to lack any or all PERV genes is produced by Clustered Regularly-Interspaced Short Palindromic Repeats (CRISPR)-Cas9 editing.

In certain embodiments of the above described methods, either one or both of (i) the population of mammalian pluripotent cells is obtained from a source that is selected from embryonic cells, umbilical cord cells, placental cells, neural crest progenitors, adult tissue stem cells, and somatic tissue cells; and (ii) the population of mammalian pluripotent cells is cultured in a culture medium that comprises one or more *in vitro* CP differentiation agents selected from a bone morphogenic protein (BMP) or a BMP signaling pathway agonist, a transforming growth factor-beta (TGF-β) superfamily member or a TGF-β signaling pathway agonist, a nodal protein or a nodal signaling pathway agonist, a mammalian growth and differentiation factor (GDF) or a GDF signaling pathway agonist, a Wnt protein ligand or a Wnt signaling pathway agonist, a fibroblast growth factor (FGF) or an FGF signaling pathway agonist, and sonic hedgehog (Shh) or a Shh signaling pathway agonist, under conditions and for a time sufficient to obtain said plurality of *in vitro* differentiated choroid plexus (CP) cells.

In certain further embodiments the Wnt signaling pathway agonist is selected from WAY-316606 (SFRP inhibitor), IQ1 (PP2A activator), QS11 (ARFGAP1 activator), 2-amino-4-[3,4-(methylenedioxy) benzyl-amino]-6-(3-methoxyphenyl) pyrimidine, Norrin, R-spondin-1, R-spondin-2, R-spondin-3, or R-spondin-4, lithium chloride, lithium carbonate, lithium citrate, lithium orotate, lithium bromide, lithium fluoride, lithium iodide, lithium acetate, lithium hydroxide, lithium aluminum hydride, lithium perchlorate, lithium nitrate, lithium diisopropylamide, lithium borohydride, lithium oxide, lithium sulfate, lithium hexafluorophosphate, lithium tetroxide, lithium sulfide, lithium hydride, lithium amide, lithium lactate, lithium tetrafluoroborate, lithium dimethylamide, lithium phosphate, lithium peroxide, lithium manganese oxide, lithium methoxide,

lithium metaborate, lithium stearate, or another lithium salt that comprises cationic lithium.

In certain embodiments of methods described above, the pericyte stressor comprises one or more agents selected from hydrogen peroxide, nitric oxide, tert-butylhydroperoxide, heavily-oxidized glycated LDL, and a pro-apoptotic agent. In certain embodiments of methods described above the level of pericyte loss comprises a level of one or more of pericyte cell death, pericyte apoptosis, pericyte necrosis, and pericyte autophagy. In certain embodiments of methods described above the level of pericyte dysfunction comprises a level of one or more of reactive oxygen species (ROS) production, reactive nitrogen species (RNS) production, matrix metalloproteinase 2 (MMP2) production, matrix metalloproteinase 9 (MMP9) production, angiopoietin 1 production, fibronectin 1 production, platelet derived growth factor receptor beta (PDGFRβ) expression, connexin 43 expression, NG2 expression, and IL-17R(A/C) expression. In certain embodiments the candidate PPA is a cerebrospinal fluid (CSF) component that is produced by choroid plexus (CP) cells.

In certain embodiments of methods described above, prior to being cultured, the human CNS pericyte is obtained from a human having one of Parkinson's disease, Alzheimer's disease, Huntington's disease, and a retinal degenerative disease. In certain further embodiments the retinal degenerative disease is selected from macular degeneration, diabetic retinopathy, and retinitis pigmentosa.

In certain other embodiments according to the present disclosure, there is provided a screening method for identifying a pericyte protective agent (PPA) that protects a peripheral nervous system (PNS) pericyte from a pericyte stressor, wherein said pericyte stressor is capable of inducing at least one of PNS pericyte loss and PNS pericyte dysfunction, the method comprising (a) contacting, simultaneously or sequentially and in any order, (i) a cultured human PNS pericyte, (ii) the pericyte stressor that is capable of inducing at least one of PNS pericyte loss and PNS pericyte dysfunction, (iii) a conditioned culture medium that has been conditioned by mammalian choroid plexus (CP)

cells and that contains a pericyte protective agent (PPA) produced by said CP cells, or an isolated fraction of said conditioned culture medium that contains the PPA, which PPA is capable of decreasing in the cultured human PNS pericyte a level of at least one of PNS pericyte loss and PNS pericyte dysfunction, relative to the level that is detected when the PPA is absent, and (iv) one or a plurality of antibodies that have been generated against a portion of the culture medium that has been conditioned by mammalian choroid plexus (CP) cells and which contains the pericyte protective agent (PPA) produced by said CP cells, under conditions and for a time sufficient to induce detectable PNS pericyte loss or detectable PNS pericyte dysfunction when the PPA is absent, thereby to obtain a human PNS pericyte test culture; (b) detecting, in the human PNS pericyte test culture of (a), a level of at least one of PNS pericyte loss and PNS pericyte dysfunction that is decreased to a lesser degree relative to the level that is detected when the one or plurality of antibodies is absent, thereby indicating that the one or plurality of antibodies is capable of neutralizing the PPA; and (c) isolating the PPA by binding to at least one of the antibodies that is capable of neutralizing the PPA.

In certain further embodiments of any of the above described embodiments, the PPA comprises an H_2O_2-sensitive, thermostable, hexane-insoluble/ diethyl ether-insoluble/ ethyl acetate-insoluble and water-soluble molecule having a molecular weight of less than 3 kDa that is obtained in a culture medium that has been conditioned by cultured mammalian choroid plexus (CP) cells, wherein the PPA is capable, upon being contacted with CNS pericytes, of decreasing a level of CNS pericyte loss or CNS pericyte dysfunction, relative to the level of CNS pericyte loss or CNS pericyte dysfunction when the PPA is absent. In other embodiments the present disclosure provides a pericyte protective agent (PPA) which comprises an H_2O_2-sensitive, thermostable, hexane-insoluble/ diethyl ether-insoluble/ ethyl acetate-insoluble and water-soluble molecule having a molecular weight of less than 3 kDa that is obtainable from a culture medium that has been conditioned

by cultured mammalian choroid plexus (CP) cells, wherein the PPA is capable, upon being contacted with CNS pericytes, of decreasing a level of CNS pericyte loss or CNS pericyte dysfunction, relative to the level of CNS pericyte loss or CNS pericyte dysfunction when the PPA is absent.

These and other aspects of the herein described invention embodiments will be evident upon reference to the following detailed description and attached drawings. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign (non-U.S.) patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet are incorporated herein by reference in their entirety as if each was incorporated individually. Aspects and embodiments of the invention can be modified, if necessary, to employ concepts of the various patents, applications and publications to provide yet further embodiments.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Figures 1a - 1b show that CP capsules and CP capsule-conditioned media attenuated oxidative stress-induced death of cultured human CNS pericytes.

Figures 2a - 2b show that CP capsule-conditioned media attenuated oxidative stress-induced death of cultured human CNS pericytes.

Figures 3A - 3J show exemplary CP products that may occur as CSF components.

Figure 4 shows a summary of the physicochemical properties of the pericyte protective agent (PPA) present in CP capsule-conditioned media (CPe), as characterized by PPA activity in CPe (protective), by PPA activity in a low molecular weight (MW < 3kDa) fraction of CPe, by thermostable PPA

activity following CPe reflux, and by PPA activity in water soluble but not non-polar organic solvent-extracted CPe fractions.

DETAILED DESCRIPTION

The present invention is directed in certain embodiments as described herein to compositions and methods for treating nervous system diseases or disorders, including neurodegenerative and other neurological diseases. In particular embodiments and as described herein for the first time, there are provided methods for identifying a pericyte protective agent (PPA) that protects a central nervous system (CNS) pericyte from a pericyte stressor that is capable, in the absence of the PPA, of inducing CNS pericyte loss and/or CNS pericyte dysfunction.

The present disclosure relates in part to the unexpected observation that cultured human CNS pericytes are protected against stressor-induced CNS pericyte loss and/or CNS pericyte dysfunction by PPA that are produced by choroid plexus (CP) –derived compositions, including CP products previously thought to act directly on neuronal cells as neuroprotectants.

More specifically and as described in greater detail below, one or more PPA present in conditioned culture medium obtained from encapsulated xenogeneic CP cells protected cultured human CNS pericytes against pericyte loss induced by the exemplary pericyte stressor, hydrogen peroxide.

CP-derived pericyte protective agents (PPA) identified by the presently disclosed methods thus may find uses in treating CNS disease. The contemplated embodiments need not, however, be so limited. For example, the screening methods described herein will also find other uses, such as in the screening of candidate PPAs obtained not only from CP cells but also from other sources, including synthetic or other candidate PPAs. Certain embodiments further envision use of the presently disclosed screening assays to identify synthetic small molecules or other mimetics that mimic a PPA that

has initially been identified as a CP product according to the methodologies described herein.

As disclosed herein, cultured human CNS pericytes can be protected from stressor agents that would otherwise induce CNS pericyte loss (*e.g.*, cell death, including cell death by any of a number of known mechanisms, such as apoptosis, autophagy, necrosis, ferroptosis, necroptosis, etc.) or CNS pericyte dysfunction (*e.g.*, onset of a pericyte phenotype associated with a CNS pathology such as that of a neurodegenerative disease). Protection of CNS pericytes, a non-neuronal cell type, can be surprisingly achieved by exposing the CNS pericytes to pericyte protective agents (PPA) that are produced by mammalian choroid plexus (CP) cells, and in particular, PPA that are produced by appropriately selected non-immunogenic encapsulated xenogeneic and/or allogeneic choroid plexus (CP) cells as described herein.

Accordingly, also provided herein are methods for identifying a pericyte protective agent (PPA) that protects a CNS pericyte from a pericyte stressor, where the so-identified PPA may be usefully administered to treat, reduce (*e.g.*, decrease in a statistically significant manner relative to an appropriate control) the severity of, or the likelihood of occurrence of, a CNS condition associated with CNS pericyte loss or CNS pericyte dysfunction, such as a neurodegenerative disease.

Certain other particular embodiments as disclosed herein contemplate methods for identifying a pericyte protective agent (PPA) that protects a peripheral nervous system (PNS) pericyte from a pericyte stressor that is capable, in the absence of the PPA, of inducing PNS pericyte loss and/or PNS pericyte dysfunction. Hence, although throughout the present disclosure there are presented various descriptions of protection of CNS pericytes from a CNS pericyte stressor, references herein to CNS pericytes and/or to a CNS pericyte stressor are intended in certain of the presently contemplated embodiments also to refer in the alternative to PNS pericytes and/or to a PNS pericyte stressor.

It will therefore be understood that certain embodiments may relate to protection of PNS pericytes and thus the presently disclosed methods as exemplified using CNS pericytes may in the alternative be practiced with PNS pericytes and PNS pericyte stressors to identify PPA for PNS pericytes. These and related embodiments may find uses in treating diseases and disorders of the PNS. Accordingly, references made herein to CNS pericytes and CNS pericyte protection may, in certain such related but distinct alternative embodiments, also apply to PNS pericytes and protection thereof. In view, however, of certain differences recognized by the art between CNS pericytes and PNS pericytes, and between certain CNS diseases and disorders and PNS diseases and disorders, it will also be understood that some references made herein to CNS pericytes and CNS pericyte protection may not, in certain such related but distinct alternative embodiments, also apply to PNS pericytes and protection thereof.

The present disclosure thus describes determination of a CNS pericyte protective effect, and in certain preferred embodiments a protective effect that is conferred by one or more pericyte protective agent (PPA) produced by isolated (*e.g.*, removed or separated from the biological tissue environment in which they occur naturally), optionally encapsulated CP cells. The PPA may be found in culture medium conditioned by CP cells cultured according to any of a variety of established methodologies (*e.g.,* Thanos et al., 2007 *Tiss. Eng.* 13:747; Angelow et al., 2004 *Adv. Drug Deliv. Rev.* 56:1859; Haselbach et al., 2001 *Microsc. Res. Tech.* 52:137; Balusu et al., 2016 *EMBO Mol. Med.* 8:1162; Huang et al., 2014 *Neurosci. Lett.* 566:42; Emerich et al., 2007 *Cell Transplant.* 16:697) including CP cells cultured on permeable membrane supports and/or CP cell-containing capsules produced and selected as described herein and also according to WO 2016/187067 and US 2016/0361365, including the composition and size of the capsules, the source, preparation and number of cells that are contained therein, optional induction of CP cells with one or more CP inducing agents, and the determination of

cytoprotective effects of CP products on cultured human CNS pericytes. CP cells may be of human, porcine, ovine, bovine, caprine, or non-human primate or other mammalian origin, and in certain non-limiting embodiments may be obtained from fetal or neonatal choroid plexus tissue by techniques with which those skilled in the art will be familiar.

In particular and as described herein, CP conditioned medium contains one or more PPA responsible for protecting human CNS pericytes against pericyte stressor-induced CNS pericyte loss and/or stressor-induced CNS pericyte dysfunction. For example and according to certain herein disclosed embodiments, the PPA comprises an H_2O_2-sensitive, thermostable, hexane-insoluble/ diethyl ether-insoluble/ ethyl acetate-insoluble and water-soluble molecule having a molecular weight of less than 3 kDa that is obtained in a culture medium that has been conditioned by cultured mammalian choroid plexus (CP) cells, wherein the PPA is capable, upon being contacted with CNS pericytes, of decreasing a level of CNS pericyte loss or CNS pericyte dysfunction, relative to the level of CNS pericyte loss or CNS pericyte dysfunction when the PPA is absent. Certain of the presently disclosed embodiments thus represent new and useful improvements to the use of cultured CP cells and/or CP encapsulation for the identification of treatments for CNS disease, which improvements could not have been predicted from previous knowledge in the art.

As disclosed herein for the first time, after culturing selected isolated and in certain embodiments encapsulated xenogeneic and/or allogeneic choroid plexus (CP) cells in a culture medium, a CP cell-conditioned medium is obtained that comprises one or more pericyte protective agents (PPA) having demonstrable activity in the protection of cultured human CNS pericytes against the deleterious effects of a pericyte stressor as provided herein. In particular, the PPA may protect pericytes against stressor-induced CNS pericyte loss or CNS pericyte dysfunction. CSF components may be produced constitutively by such cells and/or may be produced by such cells at a

level that is altered (*e.g.*, increased or decreased in a statistically significant manner relative to the level prior to or in the absence of contact with a CP inducing agent) as described in WO 2016/187067 and US 2016/0361365, and which for certain preferred CSF components is greater than the level at which the CP cells produce the CSF component(s) without being contacted with the choroid plexus inducing agent.

Certain preferred embodiments relate to an *in vitro* screening assay that permits identification of a pericyte protective agent (PPA) that protects a CNS pericyte from a pericyte stressor which, as provided herein, is capable of inducing CNS pericyte loss (*e.g.*, pericyte cell death) and/or CNS pericyte dysfunction (*e.g.*, loss of BBB maintenance capability, decreased PDGF-R expression causing altered PDGF signaling responses, inappropriate neuroinflammatory response to pro-inflammatory stimuli, etc.).

In these and related embodiments, cultured human CNS pericytes may be contacted with the pericyte stressor as provided herein in the absence and presence of a candidate pericyte protective agent (PPA), under conditions and for a time sufficient for the stressor to induce detectable CNS pericyte loss and/or detectable CNS pericyte dysfunction when the PPA is absent. A human CNS pericyte test culture is obtained when the candidate PPA is present: the pericyte, pericyte stressor, and candidate PPA may be contacted with one another simultaneously or sequentially and in any order to obtain the human CNS pericyte test culture.

Detectable CNS pericyte loss and detectable CNS pericyte dysfunction refer to quantifiable parameters that may be determined using any of a wide variety of art-accepted methodologies for characterizing the biological status of a CNS pericyte. Detectable CNS pericyte loss and/or detectable CNS pericyte dysfunction will thus be understood to refer to a level of the respective parameter (*e.g.*, percentage or number of viable pericytes, and/or percentage of cells expressing, or amount expressed of, a phenotype, phenotypic marker or other reporter molecule, whether released by a pericyte or retained by the

pericyte) that has been altered (*e.g.*, increased or decreased in a statistically significant manner relative to an appropriate control), for example, as a result of contact between the CNS pericyte and the pericyte stressor.

Typically, a pericyte stressor may be expected to induce a level of pericyte loss that is detectable as a statistically significant decrease in the percentage or number of viable pericytes that can be detected in the absence of the stressor, and a candidate pericyte protective agent (PPA) that exhibits PPA activity will decrease the level of stressor-induced pericyte loss in a statistically significant manner relative to the level detected when the candidate PPA is absent. Accordingly, the level of pericyte loss in the presence of a PPA is decreased in a statistically significant manner (*i.e.*, the percentage or number of viable pericytes is increased) relative to the level that is detected when the PPA is absent.

Particularly preferred for use in these and related embodiments are cultured human CNS pericytes, which may be prepared according to established methodologies, optionally with routine modifications (*e.g.*, Rustenhoven et al., 2015 *Sci. Rep.* 5:12132; Jansson et al., 2014 *J. Neuroinflamm.* 11:104; Rustenhoven et al., 2016 *J. Neuroinflamm.* 13:1; Jansson et al., 2016 *J. Neuroinflamm.* 13:1; Dieriks et al., 2017 *Sci. Rep.* 7:42984; Rustenhoven et al., 2017 *Trends Pharmacol. Sci.* 38:291; and references cited therein). As described in greater detail below, for instance, for the practice of an exemplary embodiment of the herein described method, human CNS pericytes were isolated from neurologically normal post-mortem adult human brain tissue through mechanical dissociation and enzymatic digestion, and then cultured, essentially as described by Rustenhoven et al., 2015 *Sci Rep.* 5: 12132.

Pericyte stressors capable of inducing at least one of CNS pericyte loss and CNS pericyte dysfunction include chemical, biological and physical agents having one or more of a pro-oxidant, a pro-inflammatory, a pro-apoptotic, a pro-necroptotic, a pro-ferroptotic, and a pro-autophagy effect. Non-

limiting examples of pro-oxidant pericyte stressors include hydrogen peroxide (H_2O_2), nitric oxide (NO), tert-butylhydroperoxide, heavily-oxidized glycated LDL, and hypoxia (*e.g.,* Diffley et al., 2009 *Mol. Vis.* 15:135; Barth et al., 2007 *Diabetologia* 50:2200; Song et al., 2005 *Invest. Ophthalmol. Vis. Sci.* 46:2974; Fu et al., 2016 *Diabetologia* 59:2251; Nishimura et al., 2016 *J Cereb. Blood Flow Metab.* 36:1143).

In certain preferred embodiments described herein, hydrogen peroxide is the pericyte stressor that is present in a human CNS pericyte test culture, along with a cultured human CNS pericyte and a candidate PPA. Without wishing to be bound by theory, hydrogen peroxide is believed to exert a stressor effect on CNS pericytes via pro-oxidant activity.

Pro-inflammatory pericyte stressors include but need not be limited to tumor necrosis factor-α (TNFα), interferon-γ (IFNγ), interleukin-1β (IL-1β), chemokine CCL2, bacterial lipopolysaccharide (LPS), and transforming growth factor-β1 (TGF-β1) (*e.g.,* Kose et al., 2007 *Drug Metab. Pharmacokinet.* 22:255; Persidsky et al., 2016 *J. Cereb. Blood Flow Metab.* 36:794; Jansson et al., 2014 *Jl. Neuroinflamm.* 11:104).

Pro-apoptotic pericyte stressors include but need not be limited to monensin (*e.g.,* Ketola et al., 2010 *Mol Canc Ther* 9:3175), stilbenes (*e.g.,* Tsai et al., 2017 *J. Food Drug Anal.* 25:134), quinazolines (*e.g.*, Mehndiratta et al., 2016 *Recent Pat Antcanc. Drug Discov.* 11:2), Bcl-2 family members (*e.g.*, Um, 2016 *Oncotarget* 7:5193), ruthenium (II) complexes (*e.g.,* Zhang et al., 2014 *Eur. J. Med. Chem.* 80:316) and other apoptosis-promoting agents known in the art.

Detection of CNS pericyte loss, such as detection of a level of CNS pericyte loss at one or a plurality of time points before, during, and/or following exposure to a pericyte stressor and/or a candidate PPA, may be accomplished by any of a variety of techniques with which the skilled artisan will be familiar, such as methods for determining viability of cells and/or methods for determining whether a cell has undergone a programmed cellular death

process (*e.g.*, apoptosis, necrosis/necroptosis, ferroptosis, autophagy). Colorimetric and/or fluorimetric indicators of cell viability, for instance, indicators of vital dye exclusion or of cellular metabolic activity, are well known in the art for such purposes (*e.g.,* I. Johnson and M.T.Z. Spence (Eds.), Molecular Probes Handbook – 11th Edition 2010, Molecular Probes/ Invitrogen/ Life Technologies Corp., Carlsbad, CA).

Also known in the art and readily adaptable for use to detect CNS pericyte loss and/or CNS pericyte dysfunction according to the herein described methods are specific assay methodologies to detect apoptosis (caspase-mediated programmed cell death, as may, for example, be induced by death receptor ligands, oxidative stress, or chemotherapy); ferroptosis (iron-dependent non-apoptotic cell death characterized by lipid peroxide accumulation); necrosis or necroptosis (programmed cell death that does not involve the caspases, as may be induced, for example, by radiation, chemical or microbial factors, or other clinical insults); and autophagy (cellular self-destruction characterized by delivery of cytosolic components to the lysosomal degradation compartment, as may be induced, for example, by growth factor deprivation, essential nutrient deprivation, hypoxia, or chemotherapy). See, *e.g.,* Krysko et al., 2008 *Meths. Enzymol.* 442:307-41 ("Methods for distinguishing apoptotic from necrotic cells and measuring their clearance"); Edinger et al., 2004 *Curr. Opin. Cell Biol.* 16:663-9; Fu et al., 2016 *Diabetologia* 59:2251; Archana et al. 2013 *Indian J. Cancer* 50:274-283; Dixon et al., 2012 *Cell* 149:1060; Cao et al., 2016 *Cell. Molec. Life Sci.* 73:2195; Yu et al., 2017 *J. Cell. Mol. Med.* 21:648..

For example, carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone (Z-VAD-FMK) is a broad specificity caspase inhibitor that can be used to determine whether apoptosis is present, and a number of quantitative apoptosis-detection assays are known (*e.g.,* activated caspase detection, annexin V binding to cell membranes, M30 neoantigen detection, *see, e.g.,* Archana et al. 2013 *Indian J. Cancer* 50:274-283). Similarly,

ferrostatin-1 is a non-limiting example of a specific inhibitor of ferroptosis that controls lipid reactive oxygen species (ROS) generation and thus may be used to determine a level of ferroptosis that may be present; necrostatin-1 is a non-limiting example of a specific inhibitor of necroptosis and a level of necrotic cell death may be determined by standard methods, for instance, using the CCK-8 cell viability assay offered by Dojindo Molecular Technologies (Rockville, MD); and 3-methyladenine is a non-limiting example of a specific autophagy inhibitor, and molecular markers for detecting a level of autophagy are known, including, *e.g.*, LC3B-I/II, beclin-1, and ATG-5 (autophagy-related homolog).

Detection of CNS pericyte dysfunction, such as detection of a level of CNS pericyte dysfunction at one or a plurality of time points before, during, and/or following exposure to a pericyte stressor and/or a candidate PPA, may also be accomplished by any of a variety of techniques with which the skilled artisan will be familiar. Typically, an altered (*e.g.*, increased or decreased in a statistically significant manner relative to an appropriate control) level of an indicator of CNS pericyte dysfunction may be detected in a human CNS pericyte test culture in which a cultured human CNS pericyte has been contacted with a pericyte stressor as provided herein and a candidate PPA, relative to the level that is detected when the candidate PPA is absent.

An indicator of CNS pericyte dysfunction may include one or more detectable human CNS pericyte surface markers, one or more detectable human CNS pericyte-elaborated (*e.g.*, released soluble) factors, detectable expression of one or more human CNS pericyte genes, one or more detectable human CNS pericyte inflammatory markers, or any other detectable criterion by which CNS pericyte status, activity, function, responsiveness to stimuli, capacity, or other biological property can be assessed. Persons familiar with the relevant art will be aware of any number of approaches by which to detect human CNS pericyte dysfunction, including by way of illustration and not limitation methods for determining a level of one or more of reactive oxygen species (ROS) production, reactive nitrogen species (RNS) production, matrix

metalloproteinase 2 (MMP2) production, matrix metalloproteinase 9 (MMP9) production, angiopoietin 1 production, TGF-β1 production, fibronectin 1 production, platelet derived growth factor receptor beta (PDGFRβ) expression, connexin 43 expression, NG2 expression, IL-17R(A/C) expression, α1-integrin expression, α-smooth muscle actin expression, and/or CX-43 expression.

For example, phenotypic marker expression by CNS pericytes is described in Hill et al., 2014 *J. Neuroimmune Pharmacol.* 9:591; and in Persidsky et al., 2016 *J. Cereb. Blood Flow Metab.* 36:794; other characterization of CNS pericytes can be found there and also in, *e.g.,* Rustenhoven et al., 2017 *Trend. Pharmacol. Sci.* 38:291; Winkler et al., 2014 *Brain Pathol.* 24:371; Bell et al., 2010 *Neuron* 68:409; Sweeney et al., 2016 *Nat. Neurosci.* 19:771; Nikolakopoulou et al., 2017 *PLoS One* 12(4): e0176225; Dore-Duffy et al., 2011 *Meth. Mol. Biol.* 686:49; Yamazaki et al., 2017 *Int. J. Mol. Sci.* 18:1965; Kose et al., 2007 *Drug Metab. Pharmacokinet.* 22:255; and Dohgu et al., 2005 *Brain Res.* 1038:208; *cf.* Liu et al. 2016 *J Immunol.* 197:2400 (non-CNS pericytes).

Further to the present disclosure for the first time that pericyte protective agent (PPA) activity is detectable in conditioned culture medium containing products of cultured mammalian choroid plexus (CP) cells, and in certain embodiments cultured encapsulated mammalian choroid plexus (CP) cells, there are also provided certain embodiments that contemplate screening of CP cell-conditioned culture medium (which may be readily separated from cultured CP cells or from cultured encapsulated CP cells by standard techniques such as centrifugation, sedimentation, filtration, etc.), and of PPA activity-containing fractions thereof (which may be readily separated from complete conditioned medium by standard biochemical techniques such as differential salt or solvent precipitation, extraction, gel or membrane size exclusion filtration, affinity, ion-exchange, reverse-phase, partition or hydrophobic chromatographic separation, etc.) to identify and characterize the

component(s) responsible for PPA activity. For example, and according to certain herein disclosed embodiments, the PPA comprises an H_2O_2-sensitive, thermostable, hexane-insoluble/ diethyl ether-insoluble/ ethyl acetate-insoluble and water-soluble molecule having a molecular weight of less than 3 kDa that is obtained in a culture medium that has been conditioned by cultured mammalian choroid plexus (CP) cells, wherein the PPA is capable, upon being contacted with CNS pericytes, of decreasing a level of CNS pericyte loss or CNS pericyte dysfunction, relative to the level of CNS pericyte loss or CNS pericyte dysfunction when the PPA is absent. In certain preferred embodiments the CP cell-conditioned medium contains one or more CSF components as provided herein, in which CSF components the PPA activity resides.

Persons familiar with the art will recognize that CSF components that are produced by CP cells comprise a large number of defined and well characterized peptides, proteins and other biologically active substances (*e.g.*, Redzic et al., 2005 *Curr. Topics Dev. Biol.* 71:1; see, *e.g.*, Fig. 3A-J) having defined chemical structures that may be detected using established techniques and routine methodologies. (See also, *e.g.,* WO 2016/187067 and US 2016/0361365.)

For instance, public database (*e.g.,* GenBank®, National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD) accession numbers for polynucleotide sequences encoding many CSF components that are peptides or proteins, and for the encoded amino acid sequences of such peptides or proteins, are set forth in Figure 3 (Figs. 3A-3J). Accordingly, determination of the production by CP cells of one or more specific CSF components may be achieved by any of a variety of approaches, such as by detection of CSF component-encoding gene expression by a nucleic acid hybridization-based technology, for instance, by polymerase chain reaction (PCR) amplification of CSF component-encoding RNA sequences (*e.g.*, Wang et al., 2009 *Nat. Rev. Genet.* 10(1):57); and/or by CSF component-encoding RNA or cDNA hybridization to complementary oligonucleotide or polynucleotide

sequences present in probe sequence arrays (*e.g.,* GENECHIP® arrays, Affymetrix, Santa Clara, CA) ; and/or by identification of CSF component-encoding transcription products by RNA sequencing (or "RNA-seq", *e.g.* Next Generation Sequencing (NGS) using Illumina sequencing by synthesis (SBS) chemistry, Illumina, Inc., San Diego, CA) (Bentley et al., 2008 *Nature*, 456:53); and/or by *in situ* hybridization (*e.g.,* Yin et al., 1998 *Brain Res.* 783:347; Swanger et al., 2011 *Meths. Mol. Biol.* 714:103) or by other nucleic acid detection techniques that are known in the art for determining the presence of specific nucleic acid sequences such as all or portions of any one or more of the RNA sequences or their corresponding DNA sequences encoding any of the CSF components provided herein (*e.g.*, in Fig. 3A-J).

Additionally or alternatively, determination of the production by CP cells of one or more specific CSF components (*e.g.*, of Fig. 3A-J) may be achieved by detection of peptides or proteins or related electrolytes, metabolites or catabolites that comprise such CSF components, for example by specific immunochemical, biochemical, or radiochemical assays or via other detectable indicator moieties, by liquid chromatography and/or mass spectrometry (*e.g.*, Holtta et al., 2015 *J. Proteome Res.* 14:654; Chiasserini et al. 2014 *J. Proteomics* 106:191; Naureen et al., 2014 *Childs Nerv. Syst.* 30:1155; Davidsson et al., 2005 *Dis. Markers* 21:81; Aluise et al. 2008 *Biochim. Biophys. Acta* 1782:549; Bonk et al., 2001 *Neuroscientist* 7:6; Casado et al., 2014 *Electrophoresis* 35:1181), by functional magnetic resonance imaging (fMRI, *e.g.,* Jasanoff, 2007 *Curr. Opin. Neurobiol.* 17:593; Bell et al., 2000 *Gene Therap.* 7:1259), or the like, or by other applicable detection technologies. CSF components are also described, for example, in R.A. Fishman, *Cerebrospinal Fluid in Diseases of the Nervous System*, W.B. Saunders, Philadelphia, PA, 1980; Cutler et al., 1982 *Ann. Neurol.* 11:1; and Hershey et al., 1980 *Ann. Neurol.* 8:426.

CSF COMPONENTS

Cerebrospinal fluid (CSF) is produced in the central nervous system (CNS) by choroid plexus epithelial cells, specialized ependymal cells lining the brain ventricles that are noteworthy for their polarization into basolateral and apical membrane domains that possess multiple electrolyte transport channels, and for their constitutive CSF secretory activity. CSF comprises a complex mixture of CSF molecular components that may include without limitation electrolytes, antioxidants, metabolites and mediators (*e.g.*, nicotinamide, NAD+, NADH, lycopene, monoamine metabolites, cryptoxanthin, carotenoids, F2-isoprostanes, 8-OHdG, long-chain polyunsaturated fatty acids, methylhistidine, 2-ketobutyric acid, biotin, and numerous others as known in the art), and proteins, including variably a number of growth factors, chemotactic factors, chaperone proteins, apolipoproteins, immunoglobulins, hemoglobins, enzymes, defensins, histones, keratins and other cytoskeleton-associated proteins.

CSF composition, including the CSF proteome, has been extensively characterized, and biomarkers associated with a variety of pathologies have been described (*e.g.*, Bora et al., 2012 *J. Proteome Res.* 11:3143; Whitin et al., 2012 *PLoS One* 7(11):e49724; Perrin et al., 2013 *PLoS One* 8(5):364314; Naureen et al., 2013 *Fluid Barriers CNS* 10:34; Fraisier et al., 2014 *PLoS One* 9(4):e93637).

Detection of relevant alterations (*e.g.,* statistically significant increases or decreases) in the quantitative representation of one or more CSF components is therefore known to those familiar with the art, for instance, in biological samples containing CSF obtained from human or animal tissues, and also including supernatant fluids or conditioned culture media or the like from cells (*e.g.*, CP cells) or tissues (*e.g.,* CP tissues or tissue fragments) that are capable of CSF production and that have been maintained *in vitro* under conditions and for a time sufficient to produce CSF or one or more CSF components. Accordingly and in view of the present disclosure, production of

one or more CSF components by a cultured CP cell and/or by a cultured encapsulated CP cell can be determined routinely through the use of existing methodologies.

The terms "isolated protein" and "isolated polypeptide" referred to herein means that a subject protein or polypeptide (*e.g.,* a protein or polypeptide CSF component or fragment thereof such as a fragment having PPA activity as provided herein) (1) is free of at least some other proteins or polypeptides with which it would typically be found in nature, (2) is essentially free of other proteins or polypeptides from the same source, *e.g.*, from the same species, (3) is expressed by a cell from a different species, (4) has been separated from at least about 50 percent of polynucleotides, lipids, carbohydrates, or other materials with which it is associated in nature, (5) is not associated (by covalent or noncovalent interaction) with portions of a protein or polypeptide with which the "isolated protein" or "isolated polypeptide" may be associated in nature, (6) is operably associated (by covalent or noncovalent interaction) with a polypeptide with which it is not associated in nature, or (7) does not occur in nature. Such an isolated protein or polypeptide can be encoded by genomic DNA, cDNA, mRNA or other RNA, or may be of synthetic origin according to any of a number of well-known chemistries for artificial peptide and protein synthesis, or any combination thereof. In certain embodiments, the isolated protein or polypeptide is substantially free from proteins or polypeptides or other contaminants that are found in its natural environment that would interfere with its use (therapeutic, diagnostic, prophylactic, research or otherwise). Similarly, an "isolated" non-protein or non-polypeptide molecule that may possess PPA activity may be a cellular or tissue product (*e.g.*, a biomolecule) that has been separated from some or all other biomolecules with which it would typically be found in nature.

According to certain embodiments it is contemplated that a choroid plexus inducing agent as provided in WO 2016/187067 and US 2016/0361365 may induce cultured CP tissue cells (optionally encapsulated CP

cells) or *in vitro* differentiated CP cells to produce altered (*e.g.*, increased or decreased in a statistically significant manner relative to controls), and in certain preferred embodiments increased, levels of one or more CSF components, including one or more candidate CSF components having PPA activity as described herein, such as the CP products and/or CSF components set forth in Figure 3A-J and including one or more of:

a growth factor that may be IGF-1, IGF-II, FGF-1, bFGF (FGF-2), FGF-9, FGF-12, FGF-18, TGF-β1, TGF-β2, TGF- β3, VEGF, VEGF-A, VEGF-B, VEGF-C/VEGF-2, EGF, growth hormone (GH), BMP-1, BMP-2, BMP-4, BMP-7, BMP-11, BMP-15, GDF-1, GDF-7, GDF-8, GDF-9, GDF-10, GDF-11, nerve growth factor (NGF), PEDF (pigment epithelium derived factor, also known as SerpinF1), glucagon-like peptide-1 (GLP-1), IGF2, BDNF, NT-3, NT-4, GDF-15, GDNF, connective tissue growth factor (CTGF), axotrophin, heparin-binding EGF-like growth factor (HB-EGF), platelet derived growth factor-alpha (PDGF-α), keratinocyte growth factor (KGF), or neurite growth-promoting factor-2/midkine (NEGF2);

a CSF antioxidant that may be ceruloplasmin, superoxide dismutase-1 (SOD-1), superoxide dismutase-2 (SOD-2, Mn-type), superoxide dismutase copper chaperone (CCS), DJ-1/PARK7, catalase, selenoproteins (I, M, N, P, S, T, W, X, 15kDa), glutathione S-transferase, glutathione S-transferase mu 2 (muscle), glutathione reductase, glutathione peroxidase, hydroxyacyl glutathione hydrolase or thioredoxin;

a chemotactic factor that may be alveolar macrophage-derived chemotactic factor-I (AMCF-I), AMCF-II, stromal cell-derived factor-2, chemokine (CXC motif) ligand 2, chemokines (*e.g.*, CCL8, CCL16, CCL19, CCL21, CCL25, CXCL2, CXCL4, CXCL9, CXCL12, CXCL13, CXCL14), chemokine (CXC motif) receptor-2, chemokine (CXC motif) receptor-4, a chemokine-like factor super family (*e.g.,* CKLF-3, -6, -7), or neurite growth-promoting factor-2/midkine (NEGF2); and/or

a chaperone protein that may be transthyretin, lipocalin-type prostaglandin D synthase/β-trace (L-PGDS), apolipoproteins (*e.g.,* apolipoprotein A, B, C, D, E, H, J, M, N, O, or R), lipocalin-6, lipocalin-7, lipocalin-15, cystatin B, cystatin C, cystatin EM, cystatin 11, a heat shock protein (HSP) family member, or DJ-1/PARK7.

It will be appreciated that any given CSF component may occur having an amino acid sequence as disclosed herein (*e.g.*, by accession number, or by disclosure in a reference publication incorporated by reference herein, or as known to those familiar with the art, etc.) or may be encoded by a polynucleotide sequence as disclosed herein (*e.g.,* by accession number, or by disclosure in a reference publication incorporated by reference herein, or as known in the art, etc.), and also that any given CSF component may have an amino acid sequence, or may be encoded by a polynucleotide sequence, that is at least 50, 55, 60, 65, 70, 75, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identical to, respectively, an amino acid sequence or a polynucleotide sequence as disclosed herein (*e.g.*, by accession number, or by disclosure in a reference publication, etc.) (Stevens et al., 2005 *J Mol Recognit* 18(2):150). In this regard, CSF components or coding sequences therefor that are less than 100 percent identical to a herein disclosed sequence (*e.g.,* by accession number, etc.) are contemplated as variants, where such variants may result from being the products of accumulated or acquired mutations, allelic variation, posttranslational or posttranscriptional processing, translational or transcriptional error, or the like. Variants are also contemplated where allogeneic or xenogeneic tissues are the sources of CP cells, for instance, where an allogeneic or xenogeneic homologue of a herein disclosed CSF component may be at least 50, 55, 60, 65, 70, 75, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identical to, respectively, an amino acid sequence or a polynucleotide sequence as disclosed herein (*e.g.*, by accession number, etc.).

When comparing polypeptide (amino acid) or polynucleotide sequences, two sequences are said to be "identical" if the sequence of amino acids or nucleotides in the two sequences is the same when aligned for maximum correspondence, as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign™ program in the Lasergene™ suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345; Hein J., 1990 *Unified Approach to Alignment and Phylogenes*, pp. 626; *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M., 1989 *CABIOS 5*:151; Myers, E.W. and Muller W.,1988 *CABIOS 4*:11; Robinson, E.D., 1971 *Comb. Theor 11*:105; Santou, N. Nes, 1987 M., *Mol. Biol. Evol. 4*:406; Sneath, P.H.A. and Sokal, R.R.,1973 *Numerical Taxonomy – the Principles and Practice of Numerical Taxonomy*, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J.,1983 *Proc. Natl. Acad., Sci. USA 80*:726.

Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman, 1981 *Add. APL. Math* 2:482, by the identity alignment algorithm of Needleman and Wunsch, 1970 *J. Mol. Biol.* 48:443, by the search for similarity methods of

Pearson and Lipman,1988 *Proc. Natl. Acad. Sci. USA* 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

Preferred examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al.,1977 *Nucl. Acids Res.* 25:3389, and Altschul et al., 1990 *J. Mol. Biol.* 215:403, respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity among two or more polypeptides or polynucleotides. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

In one illustrative example, cumulative scores can be calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). Extensions of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff,1989 *Proc. Natl. Acad. Sci. USA* 89:10915) alignments, (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

Alternatively, the sequences obtained from RNA sequence analysis (*e.g.*, RNA-seq, described above and in the Examples) are aligned to a reference genome. For example, RNA sequence reads for each sample can be mapped to a reference genome (*e.g.,* Ensembl Sscrofa10.2, and Database for Annotation, Visualization, and Integrated Discovery (DAVID), Samborski et al.,

Transcriptome changes in the porcine endometrium during the preattachment phase, 2013 *Biol Reprod.* 2013 Dec 12;89(6):134); Dennis et al., DAVID: Database for Annotation, Visualization, and Integrated Discovery, 2003 *Genome Biol.* 4(5):P3; Huang et al., DAVID Bioinformatics Resources: expanded annotation database and novel algorithms to better extract biology from large gene lists. 2007 *Nucleic Acids Res.* 2007 Jul; 35(Web Server issue):W169-75; Huang et al., Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources, 2009 *Nat Protoc.* 2009;4(1):44-57) using Tophat (v2.0.13) software to align RNA-seq reads to a reference genome, CuffLinks software to assemble reads that have been mapped by Tophat into potential transcripts to generate an assembled transcriptome, and CuffDiff software to accept the reads assembled from two or more different biological conditions and analyze them for differential expression of genes and transcripts under the different conditions (*e.g.*, induced versus control conditions). (See, *e.g.,* Ghosh et al., Analysis of RNA-Seq Data Using TopHat and Cufflinks. 2016 *Methods Mol. Biol.* 2016;1374:339-61). For library normalization, various methods, such as classic-fpkm, geometric, quartile or other methods can be applied (See, *e.g.,* http website: //cole-trapnell-lab.github.io/ cufflinks/cuffdiff/ #library-normalization-methods) combined with various cross-replicate dispersion estimation methods (*e.g.,* pooled, per-condition, bline, or poisson methods, See, *e.g.,* http website: //cole-trapnell-lab.github.io/cufflinks/cuffdiff/#library-normalization-methods).

By way of a non-limiting illustrative example, Differentially Expressed Genes (DEGs) may be identified using 'gene_exp.diff' output from the Cuffdiff software program. To detect DEGs between controls and 'induced' samples, two filtering processes can be applied. First, using a Cuffdiff status code, genes that only have "OK" status in each sample are obtained. Status code 'OK' indicates that each condition contains sufficient sequence reads in a locus for a reliable calculation of expression level and that the test is successful to calculate gene expression level in that sample. For the second filtering, a

two-fold change in expression level is calculated and only genes displaying more than two-fold changes between the samples being compared (control vs. induced) are selected. For ontology analysis, the selected gene list is applied to DAVID software (Huang et al. 2009 *Nat Protoc.* 2009;4(1):44-57; Huang et al. 2007 *Nucleic Acids Res.* 2007 35(Web Server issue):W169-75; Dennis et al., 2003 *Genome Biol.* 4(5):P3) to obtain a comprehensive set of functional annotations. Categories such as gene-disease association, homologue match, gene ontology, or pathway categories, etc. can be selected. DAVID then generates a functional annotation chart which lists annotation terms and their associated genes.

In certain embodiments, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polypeptide or polynucleotide sequence in the comparison window may comprise additions or deletions (*i.e.,* gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical amino acids residues or nucleic acid bases occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.,* the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that may encode a particular CSF component polypeptide as described herein. Some of these polynucleotides bear minimal sequence identity to the nucleotide sequence of the original polynucleotide sequence that encodes the CSF component polypeptide having an amino acid sequence that is disclosed herein. Nonetheless, polynucleotides that vary due to differences

in codon usage are expressly contemplated by the present disclosure. In certain embodiments, sequences that have been codon-optimized for mammalian expression are specifically contemplated.

As described herein, pericyte protective agent (PPA) activity is identified for the first time in conditioned culture medium prepared from encapsulated mammalian choroid plexus (CP) cells. Elucidation of the identity of one or more of these PPA and of the corresponding PPA structure may have value for development from such PPA of lead compounds for treating, reducing severity of, or reducing likelihood of occurrence of a CNS condition associated with CNS pericyte loss or CNS pericyte dysfunction. It is therefore contemplated that certain embodiments of the present invention will be of major value in the design and production of a synthetic mimetic of any CP product that is detectable in CP cell-conditioned medium by virtue of having PPA activity, such as a protein, peptide, nucleic acid, carbohydrate, lipid and/or other factor, or a complex thereof. High throughput screening; *i.e.,* automated testing or screening of a large number of additional candidate PPA, for example, is envisioned for screening synthetic or natural product libraries for additional compounds that are structurally related to PPA identified by the assays described herein, and that exhibit PPA activity.

For example, and according to certain herein disclosed embodiments, the PPA comprises an H_2O_2-sensitive, thermostable, hexane-insoluble/ diethyl ether-insoluble/ ethyl acetate-insoluble and water-soluble molecule having a molecular weight of less than 3 kDa that is obtained in a culture medium that has been conditioned by cultured mammalian choroid plexus (CP) cells (*e.g.,* encapsulated CP cells to obtain CPe conditioned medium), wherein the PPA is capable, upon being contacted with CNS pericytes, of decreasing a level of CNS pericyte loss or CNS pericyte dysfunction, relative to the level of CNS pericyte loss or CNS pericyte dysfunction when the PPA is absent.

Isolation from CP-conditioned medium (*e.g.*, CPe) of PPA activity-containing components having a molecular weight of less than 3 kDa may be achieved by subjecting the conditioned medium to any of a variety of available conventional molecular sizing methodologies, such as membrane ultrafiltration, gel filtration chromatography, dialysis with a 3 kDa molecular weight cut-off (MWCO) membrane, or other molecular sizing techniques with which persons skilled in the art will be familiar. Determination of the solubility properties of components of CP-conditioned medium (*e.g.*, CPe) exhibiting PPA activity may be achieved by differential extraction of the water-based (aqueous) CP-conditioned medium with organic solvents such as hexane, diethyl ether, and/or ethyl acetate, followed by PPA biological activity testing of the resulting fractions as disclosed herein. By way of illustration and not limitation, and as described in greater detail below, an exemplary PPA may be isolated from an aqueous CP-conditioned medium (*e.g.,* CPe) by molecular size fractionation to obtain soluble components with PPA activity of less than 3 kDa molecular weight, combined with ethyl acetate extraction to obtain an aqueous phase containing the PPA activity, from which contaminants may then be removed by Soxhlet extraction with methanol.

As described in greater detail in the Examples which follow, for example, an H_2O_2-sensitive PPA may be exemplified by exposure of a PPA-containing preparation to H_2O_2 (*e.g.*, 450 μM for 24 h at ambient room temperature), following which detectable PPA activity may be decreased in a statistically significant manner relative to the PPA activity of a control preparation that is not exposed to H_2O_2. The decrease associated with H_2O_2 sensitivity may manifest as, *e.g.*, a statistically significant decrease in PPA activity of at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, or 90%, or more, relative to an appropriate control.

As another non-limiting illustration, a thermostable PPA may be exemplified by exposure of a PPA-containing preparation to heating under reflux conditions (*e.g.,* heating to a boiling point and maintaining the boiling

point temperature under a cooling condenser to prevent solvent loss for 0.5, 1, 1.5, 2, 2.5 or more hours with stirring), following which detectable PPA activity is not decreased in a statistically significant manner relative to the PPA activity of a control preparation that is not exposed to such reflux conditions. A thermostable PPA may retain, in a statistically significant manner relative to an appropriate control, at least 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99%, or more of its PPA activity following exposure to reflux conditions.

Typically, and in certain preferred embodiments such as for high throughput drug screening, candidate agents (*e.g.*, candidate PPA such as candidate synthetic mimetics of PPA identified by the herein described methodologies) are provided as "libraries" or collections of compounds, compositions or molecules. Such molecules typically include compounds known in the art as "small molecules" and having molecular weights less than 10^5 daltons, preferably less than 10^4 daltons and still more preferably less than 10^3 daltons.

Candidate agents further may be provided as members of a combinatorial library, which preferably includes synthetic agents prepared according to a plurality of predetermined chemical reactions performed in a plurality of reaction vessels. For example, various starting compounds may be prepared employing one or more of solid-phase synthesis, recorded random mix methodologies and recorded reaction split techniques that permit a given constituent to traceably undergo a plurality of permutations and/or combinations of reaction conditions. The resulting products comprise a library of structurally related compounds that can be screened followed by iterative selection and synthesis procedures, such as a synthetic combinatorial library that may include small molecules as provided herein (see *e.g.*, PCT/US94/08542, EP 0774464, U.S. 5,798,035, U.S. 5,789,172, U.S. 5,751,629).

As presently disclosed, there is also contemplated in certain embodiments an immunological/immunochemical screening assay to identify pericyte protective agent (PPA) species within a mammalian choroid plexus

(CP) cell-conditioned medium having PPA activity. According to these and related embodiments, antibodies are generated against the PPA activity-containing CP cell-conditioned medium (or against a PPA activity-enriched fraction thereof) and are then tested for their effects on PPA activity in the herein described assays for CNS pericyte loss and/or CNS pericyte dysfunction. Neutralizing antibodies that block the PPA protective effect of the CP cell-conditioned medium may then be used to isolate and characterize the conditioned medium component(s) responsible for PPA activity.

In one such embodiment there is thus provided a screening method for identifying a pericyte protective agent (PPA) that protects a central nervous system (CNS) pericyte from a pericyte stressor, wherein said pericyte stressor is capable of inducing at least one of CNS pericyte loss and CNS pericyte dysfunction, the method comprising:

(a) contacting, simultaneously or sequentially and in any order, (i) a cultured human CNS pericyte, (ii) the pericyte stressor that is capable of inducing at least one of CNS pericyte loss and CNS pericyte dysfunction, (iii) a conditioned culture medium that has been conditioned by mammalian choroid plexus (CP) cells and that contains a pericyte protective agent (PPA) produced by said CP cells, or an isolated fraction of said conditioned culture medium that contains the PPA, which PPA is capable of decreasing in the cultured human CNS pericyte a level of at least one of CNS pericyte loss and CNS pericyte dysfunction, relative to the level that is detected when the PPA is absent, and (iv) one or a plurality of antibodies that have been generated against a portion of the culture medium that has been conditioned by mammalian choroid plexus (CP) cells and which contains the pericyte protective agent (PPA) produced by said CP cells, under conditions and for a time sufficient to induce detectable CNS pericyte loss or detectable CNS pericyte dysfunction when the PPA is absent, thereby to obtain a human CNS pericyte test culture;

(b) detecting, in the human CNS pericyte test culture of (a), a level of at least one of CNS pericyte loss and CNS pericyte dysfunction that is

decreased to a lesser degree relative to the level that is detected when the one or plurality of antibodies is absent, thereby indicating that the one or plurality of antibodies is capable of neutralizing the PPA; and

(c) isolating the PPA by binding to at least one of the antibodies that is capable of neutralizing the PPA.

In certain further embodiments the PPA is isolated as part of an immune complex in which the PPA is bound to the one or plurality of PPA-neutralizing antibodies. Methodologies for producing and isolating antibody-antigen immune complexes, and for further isolating antigens therefrom, are known in the art. For example, anti-immunoglobulin "secondary" antibodies may be used to isolate (*e.g.*, by affinity isolation or immunoprecipitation) immune complexes, as also may be natural or artificial immunoglobulin Fc region-binding reagents such as chemically stabilized *Staphylococcus aureus* (Cowan strain) suspensions, or Protein A derived therefrom, or *Streptococcal* Protein G, or synthetic mimetics thereof and/or other known anti-immunoglobulin reagents.

Certain embodiments of the present invention thus include PPA activity-neutralizing antibodies that specifically bind to a PPA as provided herein, such as a fraction or component of a PPA activity-containing preparation, for instance, a CP cell-conditioned culture medium having PPA activity.

The term "antibody" (Ab) as used herein includes monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments. In preferred embodiments, the herein described antibody is capable of neutralizing a PPA activity, such as PPA-mediated protection of a human CNS pericyte from pericyte stressor-induced pericyte loss or pericyte dysfunction. The term "immunoglobulin" (Ig) is used interchangeably with "antibody" herein.

The basic antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains.

Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus a variable domain (V_H) followed by three constant domains (C_H) for each of the α and γ chains and four C_H domains for μ and ε isotypes. Each L chain has at the N-terminus, a variable domain (V_L) followed by a constant domain (C_L) at its other end. The V_L is aligned with the V_H and the C_L is aligned with the first constant domain of the heavy chain (C_H1). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a V_H and V_L together forms a single antigen-binding site.

The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains (C_L). Depending on the amino acid sequence of the constant domain of their heavy chains (C_H), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, having heavy chains designated alpha (α), delta (δ), epsilon (ε), gamma (γ) and mu (μ), respectively. The γ and α classes are further divided into subclasses on the basis of relatively minor differences in C_H sequence and function, *e.g.*, humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. It will be appreciated that mammals encoding multiple Ig isotypes will be able to undergo isotype class switching.

An IgM antibody consists of 5 of the basic heterotetramer units along with an additional polypeptide called J chain, and therefore contains ten antigen binding sites, while secreted IgA antibodies can polymerize to form polyvalent assemblages comprising two to five of the basic four-chain units along with J chain. In the case of IgG, the four-chain unit is generally about 150,000 daltons in molecular weight. For the structure and properties of the

different classes of antibodies, see, *e.g.*, Basic and Clinical Immunology, 8th edition, Daniel P. Stites, Abba I. Terr and Tristram G. Parslow (eds.), Appleton & Lange, Norwalk, Conn., 1994, page 71, and Chapter 6.

The V domain mediates antigen binding and defines specificity of a particular antibody for its particular antigen. The gene sequence encoding the V_H domain has multiple copies of variable (V), diversity (D), and joining (J) segments. The gene sequence encoding the V_L domain contains multiple copies of V and J segments. The V_H and V_L regions undergo gene rearrangement (*i.e.,* somatic recombination) to develop diverse antigen specificity in antibodies. The term "variable" refers to the fact that certain segments of the V domains differ extensively in sequence among antibodies.

However, the variability is not evenly distributed across the 110-amino acid span of the variable domains. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by short regions of extreme variability called "hypervariable regions." These hypervariable regions are the result of somatic hypermutation during the affinity maturation process, and they are typically each 9-18 amino acids long. However, they have been found to range from 4-28 amino acids in length depending upon the particular epitope. For example, CDR3 regions up to at least 22 or 23 amino acids in length have been described. *See*, *e.g.*, Morea V, *et al.*, *J Mol Biol.* 275(2):269-94 (1998) and Kabat, E.A., *et al.*, Sequences of Proteins of Immunological Interest, Fifth Edition. NIH Publication No. 91-3242 (1991).

The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (*see* Kabat *et al.*, Sequences of Proteins of Immunological Interest,

5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody that are responsible for antigen binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (*e.g.*, around about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the V_L, and around about 28-36(H1), 50-65 (H2) and 95-102 (H3) in the V_H; Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and/or those residues from a "hypervariable loop" (*e.g.*, residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the V_L, and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the V_H ; Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987)).

An "isolated antibody" is one that has been separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody is purified: (1) to greater than 95% by weight of antibody as determined by the Bradford method, and most preferably more than 99% by weight; (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator; or (3) to homogeneity by SDS-PAGE under reducing or non-reducing conditions using Coomassie blue or, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

An "intact" antibody is one that comprises an antigen-binding site as well as a C_L and at least heavy chain constant domains, C_H1, C_H2 and C_H3. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variants thereof. Preferably, the intact antibody has one or more effector functions.

An "antibody fragment" is a polypeptide comprising or consisting of a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies (*see* U.S. Pat. No. 5,641,870; Zapata *et al.*, *Protein Eng*. 8(10): 1057-1062 [1995]); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (V_H), and the first constant domain of one heavy chain (C_H1). Each Fab fragment is monovalent with respect to antigen binding, *i.e.*, it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large $F(ab')_2$ fragment that roughly corresponds to two disulfide linked Fab fragments having divalent antigen-binding activity and is still capable of cross-linking antigen. Both the Fab and $F(ab')_2$ are examples of "antigen-binding fragments." Fab' fragments differ from Fab fragments by having additional few residues at the carboxy terminus of the C_H1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments that have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "Fc" fragment comprises the carboxy-terminal portions (*i.e.*, the CH2 and CH3 domains) of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region. The Fc domain is the portion of the antibody recognized by cell receptors, such as the FcR, and to which the complement-activating protein, C1q, binds.

"Fv" is the minimum antibody fragment that contains a complete antigen-recognition and antigen-binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (three loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

"Single-chain Fv" also abbreviated as "sFv" or "scFv" are antibody fragments that comprise the V_H and V_L antibody domains connected into a single polypeptide chain. Preferably, the sFv polypeptide further comprises a polypeptide linker between the V_H and V_L domains that enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994); Borrebaeck 1995, *infra.*

The term "diabodies" refers to small antibody fragments prepared by constructing sFv fragments (see preceding paragraph) with short linkers (about 5-10 residues) between the V_H and V_L domains such that inter-chain but not intra-chain pairing of the V domains is achieved, resulting in a bivalent fragment, *i.e.*, fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two "crossover" sFv fragments in which the V_H and V_L domains of the two antibodies are present on different polypeptide chains. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

As used herein, the term "polyclonal antibody" refers to an antibody obtained from a population of antigen-specific antibodies that recognize more than one epitope of the specific antigen. "Antigen" or "immunogen" refers to a peptide, lipid, polysaccharide or polynucleotide which is recognized by the adaptive immune system. Antigens may be self or non-self molecules. Examples of antigens include, but are not limited to, bacterial cell wall components, pollen, and rh factor. The region of an antigen that is specifically recognized by a specific antibody is an "epitope" or "antigenic determinant." A single antigen may have multiple epitopes.

The term "monoclonal antibody" (mAb) as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations that include different antibodies directed against different epitopes, each monoclonal antibody is directed against a single epitope of the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies useful in the present invention may be prepared by the hybridoma methodology first described by Kohler *et al.*, *Nature*, 256:495 (1975), or may be made using recombinant DNA methods in bacterial, eukaryotic animal or plant cells (*see*, *e.g.*, U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, *Nature*, 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991), for example.

The monoclonal antibodies herein include "chimeric antibodies" in which a portion of the heavy and/or light chain is identical with or homologous

to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (*see*, U.S. Pat. Nos. 4,816,567; 5,530,101 and 7,498,415; and Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). For example, chimeric antibodies may comprise human and non-human residues. Furthermore, chimeric antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. For further details, see Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). Chimeric antibodies also include primatized and humanized antibodies.

A "humanized antibody" is generally considered to be a human antibody that has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are typically taken from a variable domain. Humanization is traditionally performed following the method of Winter and co-workers (Jones *et al.*, *Nature*, 321:522-525 (1986); Reichmann *et al.*, *Nature*, 332:323-327 (1988); Verhoeyen *et al.*, *Science*, 239:1534-1536 (1988)), by substituting non-human variable sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. Nos. 4,816,567; 5,530,101 and 7,498,415) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In some instances, a "humanized" antibody is one which is produced by a non-human cell or animal and comprises human sequences, *e.g.*, H_C domains.

A "human antibody" is an antibody containing only sequences present in an antibody naturally produced by a human. However, as used herein, human antibodies may comprise residues or modifications not found in

a naturally occurring human antibody, including those modifications and variant sequences described herein. These are typically made to further refine or enhance antibody performance. In some instances, human antibodies are produced by transgenic animals. For example, *see* U.S. Pat. Nos. 5,770,429; 6,596,541and 7,049,426.

Antibody "effector functions" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptor); and B cell activation.

The phrase "functional fragment or analog" of an antibody is a compound having qualitative biological activity in common with a full-length antibody. For example, a functional fragment or analog of an anti-PPA antibody is one that can bind to a PPA and at least partially (*e.g.,* in a statistically significant manner relative to an appropriate control) neutralize the ability of the PPA to protect a cultured human CNS pericyte against pericyte stressor-induced CNS pericyte loss or CNS pericyte dysfunction as provided herein.

An antibody having a "biological characteristic" of a designated antibody is one that possesses one or more of the biological characteristics of that antibody which distinguish it from other antibodies. For example, in certain embodiments, an antibody with a biological characteristic of a designated antibody will bind the same epitope as that bound by the designated antibody and/or have a common effector function as the designated antibody.

As used herein, an antibody is said to be "immunospecific," "specific for" or to "specifically bind" an antigen if it reacts at a detectable level with the antigen, preferably with an affinity constant, K_a, of greater than or equal to about 10^4 M^{-1}, or greater than or equal to about 10^5 M^{-1}, greater than or equal to about 10^6 M^{-1}, greater than or equal to about 10^7 M^{-1}, or greater

than or equal to 10^8 M^{-1}. Affinity of an antibody for its cognate antigen is also commonly expressed as a dissociation constant K_D, and in certain embodiments, a PPA-specific antibody specifically binds to a PPA if it binds with a K_D of less than or equal to 10^{-4} M, less than or equal to about 10^{-5} M, less than or equal to about 10^{-6} M, less than or equal to 10^{-7} M, or less than or equal to 10^{-8} M. Affinities of antibodies can be readily determined using conventional techniques, for example, those described by Scatchard *et al.* (*Ann. N.Y. Acad. Sci. USA* 51:660 (1949)).

Binding properties of an antibody to antigens, cells or tissues thereof may generally be determined and assessed using immunodetection methods including, for example, immunofluorescence-based assays, such as immuno-histochemistry (IHC) and/or fluorescence-activated cell sorting (FACS).

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as polysorbate 20 (TWEEN™) polyethylene glycol (PEG), and poloxamers (PLURONICS™), and the like.

CP CELL SOURCES

Mammalian choroid plexus (CP) cells may be of human, porcine, ovine, bovine, caprine, or non-human primate or other mammalian origin, and in

certain non-limiting embodiments may be obtained from fetal or neonatal choroid plexus tissue by techniques known to those familiar with the art.

In certain embodiments CP tissue fragments may be prepared and encapsulated in CP cell-containing capsules selected as described in previous teachings directed to CP xenotransplantation. General methodologies for the preparation and use of such capsules are described, for example, in US6322804, US5834001, US6083523, US2007/134224, US5869463, US2004/213768, US2009/0214660, US2009/0047325, US2007/134224, US2004/213768, US2005/0265977, US6083523, and US2009/0047325 and related patent application publications. US2009/0047325 describes an exemplary preparation of neonatal CP cells for xenotransplantation. In certain other embodiments, however, the CP cells are non-encapsulated.

CP cells, whether encapsulated or non-encapsulated, may be cultured according to any of a variety of established methodologies including those cited above and also as described in, *e.g.,* Thanos et al., 2007 *Tiss. Eng.* 13:747; Angelow et al., 2004 *Adv. Drug Deliv. Rev.* 56:1859; Haselbach et al., 2001 *Microsc. Res. Tech.* 52:137; Balusu et al., 2016 *EMBO Mol. Med.* 8:1162; Huang et al., 2014 *Neurosci. Lett.* 566:42; Emerich et al., 2007 *Cell Transplant.* 16:697). Preferably CP cells are cultured in culture medium under conditions that permit release of PPA by CP cells into the culture medium, to obtain a CP cell-conditioned medium.

In certain embodiments CP cells may be cultured on permeable membrane supports such as TransWell™ tissue culture inserts, which may permit the CP cells to be co-cultured in a spatially separate compartment that is nevertheless in fluid communication with cultured human pericytes. In such embodiments, PPA released by the CP cells into the culture medium can traverse the permeable membrane to enter the compartment in which the pericytes are cultured, in order for the PPA to contact the pericytes.

In certain other embodiments CP cells may be encapsulated and cultured in CP cell-containing capsules as described in one or more of, *e.g.*, US

2009/0047325; US 8,129,186, US 2009/0214660, US 6,322,804; US 6,083,523, US 5,753,491), US 8,748,176; Watanabe et al., 2012 *Jl. Neurosci.* 32(45):15934, US 2007/0134224, US 4,892,538, and US 2012/0003190. Alternatively, CP cells may be processed and encapsulated in biocompatible permeable capsules that may be produced and selected as described in WO 2016/187067 and US 2016/0361365, optionally with induction of CP cells with one or more CP inducing agents as described therein. Identification of PPA activity in conditioned medium from such capsules can then be made according to the methods described herein, for instance, by the determination of cytoprotective effects of released CP products on cultured human CNS pericytes. Certain of the presently disclosed embodiments thus relate to biocompatible, non-immunogenic, semi-permeable alginate capsules containing PPA-producing xenogeneic and/or allogeneic CP cells for maintenance in culture to generate PPA-containing conditioned medium.

With respect to the biological sources of CP tissues and/or CP cells, however, the present embodiments are not intended to be so limited, such that there are also presently contemplated embodiments in which mammalian choroid plexus (CP) tissue may be obtained from a mammal that is xenogeneic relative to the source of the cultured pericytes being contacted with a pericyte stressor as provided herein. CP tissue from which CP cell cultures (optionally encapsulated) are prepared to generate CP cell-conditioned culture media may thus be obtained from porcine, ovine, bovine, caprine, non-human primate, or other mammalian sources. In certain other embodiments the CP cells may be obtained from a biological source that is allogeneic to the subject undergoing treatment, *e.g.*, the CP source may be tissue from a non-genetically identical individual of the same species as the subject.

In certain illustrative exemplary embodiments, allogeneic or xenogeneic pluripotent cells that are capable of differentiation into CP cells may be cultured *in vitro* under conditions and for a time sufficient to obtain a plurality of *in vitro* differentiated CP cells. Conditions for *in vitro* generation of human

CP cells from human embryonic stem cells (ESC), and of mouse CP cells from murine ESC, are described, by way of example, in Watanabe et al., 2012 *J. Neurosci.* 32(45):15934 and Sternberg et al., 2014 *Regen Med* 9(1):53. Pluripotent cells for use in these and related embodiments may comprise embryonic cells such as embryonic stem cells, embryonic stem cell-derived clonal embryonic progenitor cell lines, neural crest progenitors and/or may also comprise one or more of non-embryonic cells, such as umbilical cord cells, placental cells, dental pulp cells, adult tissue stem cells and/or mesenchymal stem cells from somatic tissues, for which methods of preparation will be known to those skilled in the relevant art (*e.g.*, Loeffler et al., 2002 *Cells Tissues Organs* 171(1):8-26).

In these and related embodiments, pluripotent cells may be cultured in a culture medium that comprises one or more *in vitro* CP differentiation agents such as any of the *in vitro* CP differentiation agents disclosed in WO 2016/187067 and US 2016/0361365. For example, pluripotent cells may be cultured in a culture medium that comprises one or more of a bone morphogenic protein (BMP) or a BMP signaling pathway agonist, a transforming growth factor-beta (TGF-β) superfamily member or a TGF-β signaling pathway agonist, a mammalian growth and differentiation factor (GDF) or a GDF signaling pathway agonist, VEGF, a Wnt protein ligand or a Wnt signaling pathway agonist, sonic hedgehog (Shh), a Shh signaling pathway agonist (*e.g.*, a synthetic small molecule agonist such as purmorphamine and/or SAG, see Stanton et al. 2009 *Mol. BioSyst* 6:44), and a fibroblast growth factor (FGF) or an FGF signaling pathway agonist, under conditions and for a time sufficient to obtain said plurality of *in vitro* differentiated choroid plexus (CP) cells (see, *e.g.*, Watanabe et al., 2012 *Jl. Neurosci.* 32(45):15934; Sternberg et al. 2014, *Regen Med*, 9(1):53; see also, *e.g.,* Ward et al. 2015 *Neuroscience* S0306-4522(15)00415; Liddelow, 2015 *Front. Neurosci.* 9 (32):1); Huang et al. 2009 *Development* 340(2):430); Schober et al., 2001 *J Comp Neurol* 439(1):32).

For example, a Wnt signaling pathway agonist may comprise one or more of WAY-316606 (SFRP inhibitor, 5-(phenylsulfonyl)-*N*-4-piperidinyl-2-(trifluoromethyl)benzene sulfonamide hydrochloride, Bodine et al., 2009 *Bone* 44:1063), IQ1 (PP2A activator, Miyabayashi et al., 2007 *Proc. Nat. Acad. Sci. USA* 104:5668), QS11 (ARFGAP1 activator, Zhang et al., 2007 *Proc. Nat. Acad. Sci. USA* 104:7444), (hetero)arylpyrimidine or 2-amino-4-[3,4-(methylenedioxy) benzyl-amino]-6-(3-methoxyphenyl) pyrimidine, Norrin (*e.g.,* Ohlmann et al., 2012 *Prog. Retin Eye Res.* 31:243; Rey et al., 2010 *Dev. Dyn* 239:102; *erratum* 2010 *Dev. Dyn.* 239:1034; GenBank Acc. No. NM_000266), R-spondin-1 (*e.g.,* Peng et al., 013 *Cell Rep.* 3:1885; GenBank Acc. No. NM_001038633; NM_001242910.1), R-spondin-2 (*e.g.,* GenBank Acc. No. NM_178565; NM_178565.4; NM_001282863.1), R-spondin-3 (*e.g.,* GenBank Acc. No. NM_032784), and R-spondin-4 (*e.g.,* GenBank Acc. No. NM_001029871.3). A Wnt signaling pathway agonist may also, in certain embodiments, comprise any suitable lithium salt, *i.e.,* a lithium compound that comprises cationic lithium and that can be contacted with cells with no or minimal toxicity, for example, lithium chloride, lithium carbonate, lithium citrate, lithium orotate, lithium bromide, lithium fluoride, lithium iodide, lithium acetate, lithium hydroxide, lithium aluminum hydride, lithium perchlorate, lithium nitrate, lithium diisopropylamide, lithium borohydride, lithium oxide, lithium sulfate, lithium hexafluorophosphate, lithium tetroxide, lithium sulfide, lithium hydride, lithium amide, lithium lactate, lithium tetrafluoroborate, lithium dimethylamide, lithium phosphate, lithium peroxide, lithium manganese oxide, lithium methoxide, lithium metaborate, lithium stearate, or any other lithium salt as may be known to those skilled in the relevant art.

Certain other preferred embodiments contemplate encapsulated choroid plexus tissue fragments that are prepared from tissue that is xenogeneic relative to the pericytes being cultured and stressed and/or to the subject undergoing treatment. For example, for the testing of human pericytes (*e.g.,* CNS pericytes) or the treatment of humans it is envisioned that

xenogeneic cultured CP cells as a source of candidate PPA, preferably encapsulated CP cells according to certain embodiments, may be obtained from a non-human source, preferably a non-human mammalian source. In certain such embodiments the non-human mammalian source of CP tissue containing CP cells that are cultured and optionally encapsulated in semi-permeable biocompatible (*e.g.*, alginate) capsules, for purposes of elaborating candidate PPA into culture medium, may be porcine tissue. Certain further embodiments relate to neonatal porcine CP tissue as the source of CP cells to be encapsulated for use in the present methods, where "neonatal" may be understood to include tissue that is obtained at any time from immediately after birth until up to three months of age.

According to certain embodiments of the present disclosure there are also provided surprising advantages that derive from the use of fetal or neonatal CP tissue (such as fetal or neonatal porcine CP tissue) that is substantially free of human pathogens, and in particular that may be substantially free of human-tropic transmissible porcine endogenous retroviruses (PERVs). It is to be understood that "substantially free" refers to a situation where conventional means for detecting human pathogens or conventional means for detecting human-tropic transmissible PERVs fail to detect such pathogens or PERVs in a statistically significant manner and with a degree of confidence of at least 95%, 96%, 97%, 98% or 99%.

In this regard, PERVs represent a serious health and safety risk accompanying the use of porcine tissues and cells for xenotransplantation into humans, despite many characteristics that make porcine tissues and cells well-suited for such transplants. In particular, PERVs that may be present in porcine donor cells to be used for transplantation are capable of infecting human cells (Fishman, 1998 *Ann. NY Acad. Sci.* 862:52; Elliott et al., U.S. 8,088,969; Park et al., 2008 *J. Microbiol. Biotechnol.* 18:1735; Hector et al. 2007 *Xenotransplant.* 14:222). By contrast, on Auckland Island, New Zealand, there has been identified a population of domesticated pigs (*Sus scrofa domesticus*)

that has been shown to be pathogen-free by a set of defined criteria, and that had missing from their genomes a full length PERV-C locus that had previously been associated with the ability of PERV to infect human cells (Garkavenko et al., 2008 *Cell Transplant.* 17:1381; Hector et al. 2007 *Xenotransplant.* 14:222). The pathogen-free animals included a subset of pigs that lacked a PERV-C *env* gene which is capable of recombination with a PERV-A *env* gene (*Id.*).

Accordingly, it is contemplated that in the practice of certain embodiments of the present disclosure, the xenogeneic tissue source of CP cells, which are cultured to produce PPA and are optionally present in semi-permeable biocompatible capsules, will comprise fetal or neonatal porcine CP tissue that is substantially free of human-tropic PERVs. In certain further embodiments the CP tissue is obtained from an animal that lacks a PERV-C *env* gene which is capable of recombination with a PERV-A *env* gene or that has been genetically engineered to lack any or all PERV genes using an established gene editing technique such as Clustered Regularly-Interspaced Short Palindromic Repeats (CRISPR)-Cas9 editing (*e.g.*, Jinek et al., 2012 *Science* 337:816; Doudna et al., 2014 *Science* 346:1258096).

In general, the materials, methods and techniques for CP encapsulation that may be employed to practice certain of the presently disclosed embodiments, may be achieved by incorporating the improvements described in WO 2016/187067 and US 2016/0361365 into adaptations of the teachings relating to choroid plexus tissue and cell preparations, to semi-permeable biocompatible capsules such as alginate capsules and the like, as may be found in one or more of the publications of Elliott and colleagues (*e.g.*, US 2009/0047325; US 8,129,186), Vasconcellos et al. (*e.g.*, US 2009/0214660), Dionne et al. (*e.g.*, US 6,322,804; US 6,083,523), Major et al. (*e.g.*, US 5,753,491), Monuki et al. (*e.g.*, US 8,748,176; see also Watanabe et al., 2012 *Jl. Neurosci.* 32(45):15934) and/or in US 2007/0134224 (Harlow et al.), US 4,892,538 (Aebischer et al.), and/or US 2012/0003190 (Yamoah et al.), all of which are incorporated by reference but which individually or in any

combination fail to teach or suggest the improvements according to the presently disclosed combinations.

According to certain embodiments, particular advantages may be obtained by including a step of selecting one or more semi-permeable biocompatible capsules (*e.g.*, alginate capsules) in which are encapsulated CP tissue fragments and/or *in vitro* differentiated CP cells according to the disclosures of US 2016/0361365 and WO 2016/187067.

Specifically and as described therein, the dimensions of the capsules to be loaded with CP cells, and the number of CP cells contained in each capsule, contribute to the CSF component production level by encapsulated cells on a per cell basis. Counterintuitively and according to non-limiting theory, increased CSF component production per cell, including CSF component per cell following induction with a CP inducing agent, was not simply and directly proportional to the number of cells present in each capsule, but was instead found to be achieved using capsules selected to have diameters of from about 400 μm to about 800 μm and typically having internal volumes of less than about one microliter, and that contained about 200 to about 10,000 cells per capsule, where "about" may be understood to represent quantitative variation that may be more or less than the recited amount by less than 50%, more preferably less than 40%, more preferably less than 30%, and more preferably less than 20%, 15%, 10% or 5%.

In certain preferred embodiments semi-permeable biocompatible capsules are thus selected that each contain at least about 200, 400, 600, 800, 1000, 2000, 3000, 4000, 5000, 7500 or 9000 and not more than about 10,000 CP cells. In certain preferred embodiments capsules are selected that each contain at least about 400, 600, 800, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, or 7500 and not more than about 8000 cells.

In certain preferred embodiments semi-permeable biocompatible (*e.g.*, alginate) capsules may be prepared that have diameters of from about

400 µm to about 800 µm, from about 500 µm to about 700 µm, from about 450 µm to about 750 µm, or from about 400 µm to about 700 µm, and that typically each have an internal volume of less than about one microliter.

Other contemplated embodiments are not so limited, however, such that capsules of any size that may contain any number of CP cells may be configured so as to be accommodated by any of a variety of cell culture vessels as may be appropriate for culturing pericytes as provided herein and/or for performing the herein described screening method for PPA. Suitable parameters will be apparent to those familiar with the art, who will be familiar with cell culture conditions and requirements for pericytes and CP cells as may be adopted in view of the present disclosure.

Selection may in certain embodiments be accomplished by any of a variety of techniques with which the skilled person will be familiar. For example, semi-permeable biocompatible capsules prepared as described herein and according to established methodologies set forth in the cited reference documents may be visualized under a microscope and manually selected according to calibrated occupancy by cells of the included volume (*e.g.*, empirically established consistent capsule occupancy, and/or by using colorimetric or fluorescent markers such as vital stains or DNA-binding dyes, etc.) using a micromanipulator, a microneedle, a microcapillary pipette, a patch-clamp device, or the like. Alternatively, automated equipment such as a large particle flow sorter (*e.g.*, COPAS™ FlowPilot™ platform, Union Biometrica Inc., Holliston, MA, USA), particle size analyzer, digital image analyzer, flow cytometer or the like may be used to process preparations of semi-permeable biocompatible capsules containing encapsulated CP cells.

In preferred embodiments, the present semi-permeable biocompatible capsules in which are "encapsulated" CP tissue fragments and/or *in vitro* differentiated CP cells include those capsules that, upon visual microscopic inspection, exhibit substantially no cells or portions of cells that are

detectable on exterior surfaces of the capsules and substantially no cells or portions of cells protruding from a capsule interior to the capsule surface.

The small enclosed volumes of the semi-permeable biocompatible (*e.g.*, alginate) capsules that are selected as disclosed herein permit efficiency and economy in the preparation and delivery of encapsulated CP cell implants, and, by virtue of the optional step of contacting with a CP inducing agent (as described in WO 2016/187067 and US 2016/0361365), further provide the ability to deliver a potent PPA source whilst occupying minimal space in a cell culture vessel.

The present disclosure is not intended to be so limited, however, such that in certain contemplated embodiments there may be a greater number of CP cells per capsule and/or capsules having different dimensions may be used and/or capsules that have not undergone the selection steps described above and in US 2016/0361365 and WO 2016/187067 may be used as sources of candidate pericyte protectant agents (PPA) as described herein. Accordingly encapsulated CP cell preparations that may be suitable for certain of the presently disclosed embodiments may include, for example, those disclosed in one or more of the publications of Elliott and colleagues (*e.g.*, US 2009/0047325; US 8,129,186), Vasconcellos et al. (*e.g.*, US 2009/0214660), Dionne et al. (*e.g.*, US 6,322,804; US 6,083,523), Major et al. (*e.g.*, US 5,753,491), Monuki et al. (*e.g.*, US 8,748,176; Watanabe et al., 2012 *Jl. Neurosci.* 32(45):15934) and/or in US 2007/0134224 (Harlow et al.), US 4,892,538 (Aebischer et al.), and/or US 2012/0003190 (Yamoah et al.).

Persons skilled in the relevant art will be familiar with any number of diagnostic, surgical and/or other clinical criteria for central nervous system conditions associated with CNS pericyte loss or CNS pericyte dysfunction, which may be employed to indicate the clinical appropriateness of, and/or to which can be adapted, administration of the pericyte protective agent (PPA) compositions described herein one or more pericyte protective agents (PPA) as

provided herein (*e.g.*, PPA identified according to the presently disclosed methods and also including mimetics of such PPA as may also be identified according to the presently disclosed methods). See, *e.g.*, Sontheimer, *Diseases of the Nervous System*, 2015 Academic Press/Elsevier, Waltham, MA; "Neurologic Disorders" in *The Merck Manual of Diagnosis and Therapy* 19th Ed. (R.S. Porter, Ed., 2011, Merck, Inc., NJ); "Neurological Diagnostic Tests and Procedures" at the website of the National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, www. ninds. nih. gov/disorders/ misc/ diagnostic_tests.htm; *Neurology in Clinical Practice* – Vol. II, 4th Edition, Bradley et al., (Eds), 2004 Butterworth Heinemann/ Elsevier, Philadelphia, PA; *Non-Neoplastic Diseases of the Central Nervous System (Atlas of Nontumor Pathology-* First Series Fascicle), D.N. Lewis et al., (eds.), 2010 Amer. Registry of Pathology, Annapolis Junction, MD; *Bradley's Neurology in Clinical Practice* (6th Ed.), R.B. Daroff et al. (eds.), 2012 Saunders/Elsevier, Waltham, MA. Criteria for diagnosis and clinical monitoring of patients having or suspected of having a CNS condition associated with CNS pericyte loss or CNS pericyte dysfunction are thus well known to those skilled in the relevant art.

Accordingly, it is contemplated that the herein described compositions and methods may find beneficial uses in a wide range of nervous system conditions and diseases for which the presence of or risk for having such a disease or condition in a subject will be apparent to the skilled clinician. Non-limiting examples of nervous system diseases to be treated according to the teachings found herein therefore include, *e.g.*, Parkinson's disease, multiple system atrophy-Parkinson type, multiple system atrophy-cerebellar type, progressive supranuclear palsy, dementia with Lewy bodies, essential tremor, drug-induced Parkinsonism, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), prion disease, motor neuron disease, spinocerebellar ataxia, spinal muscular atrophy, static nervous diseases such as stroke, CNS trauma, seizure disorders including epilepsy; progressive

neurodegenerative diseases including those associated with aging and dementia, such as Alzheimer's disease, Parkinson's disease, etc.; diseases of motor neurons and neuromuscular junctions; Huntington's disease; multiple sclerosis; CNS tumors, especially brain tumors, including neuroblastoma, glioma, astrocytoma; infectious diseases of the nervous system including meningitis, botulism, tetanus, neurosyphilis, poliomyelitis, rabies, HIV/AIDS, prion diseases, *Naegleria fowleri (*amoebic brain infection*)*; neurocysticerosis; neuropsychiatric diseases including depression, mood disorders; obsessive-compulsive disorder, schizophrenia; diseases associated with or characterized by one or more of neuronal death, glutamate toxicity, protein aggregates and/or deposits (*e.g.,* amyloid plaque formation), mitochondrial dysfunction including reactive oxygen species (ROS) production levels in excess of those found in normal, healthy control subjects; brain derived neurotrophic factor-related disorders, and other nervous system diseases including retinal degenerative diseases such as macular degeneration, diabetic retinopathy, and retinitis pigmentosa.

In certain embodiments there is thus provided a method for treating, reducing severity of, or reducing likelihood of occurrence of a CNS condition associated with CNS pericyte loss or CNS pericyte dysfunction in a subject, including, for example, by administering a PPA as presently disclosed. For example, these and related embodiments contemplate a method of treating a subject known to have or suspected of having a nervous system disease wherein the nervous system disease may be at least one of Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS, otherwise known as Motor neurone disease), progressive bulbar palsy, progressive muscular atrophy, dementia with Lewy bodies, multiple system atrophy, spinocerebellar ataxia type 1 (SCA 1), or an age-related neurodegenerative disorder. The encompassed embodiments are not intended to be so limited, however, such that methods are also contemplated of treating other neurodegenerative diseases that are characterized by CNS

pericyte loss or CNS pericyte dysfunction, in either case often also accompanied by the death of CNS neurons.

In certain embodiments there is provided a method of treating a subject known to have or suspected of having a CNS condition associated with CNS pericyte loss or CNS pericyte dysfunction, wherein the CNS condition or CNS disease is characterized by an altered level (*e.g.*, a level that is decreased or increased in a statistically significant manner relative to an appropriate control) of at least one CNS pericyte function, relative to the level of the CNS pericyte function in a control subject known to be free of the nervous system disease, including, for example, by administering a PPA as presently disclosed. For example, these and related embodiments contemplate a method of treating a subject known to have or suspected of having a CNS condition associated with CNS pericyte loss or CNS pericyte dysfunction which may be at least one of Parkinson's disease (in which an altered level of CNS pericyte loss and/or CNS pericyte dysfunction has been implicated, along with a decrease in the level of function of dopaminergic neurons), Alzheimer's disease (in which an altered level of CNS pericyte loss and/or CNS pericyte dysfunction has been implicated, along with a decrease in the level of function of noradrenergic neurons, see, *e.g.,* Adori et al. 2015, *Acta Neuropathol* 129(4):541), Huntington's disease (in which an altered level of CNS pericyte loss and/or CNS pericyte dysfunction has been implicated, along with a decrease in the level of function of medium spiny GABA neurons, (MSN)), amyotrophic lateral sclerosis (ALS, in which an altered level of CNS pericyte loss and/or CNS pericyte dysfunction has been implicated, along with a decrease in the level of function of motor neurons), and depression (in which an altered level of CNS pericyte loss and/or CNS pericyte dysfunction has been implicated, along with a decrease in the level of function of serotoninergic neurons or an altered level of maintaining the integrity of the blood brain barrier).

In certain embodiments there is provided a method of treating, reducing the severity of, or reducing the likelihood of occurrence of disease in a subject known to have or suspected of having a CNS condition associated with CNS pericyte dysfunction, wherein the CNS condition or CNS disease is characterized by an altered (*e.g.,* decreased or increased in a statistically significant manner relative to an appropriate control) level of at least one CNS pericyte function, relative to the level of the CNS pericyte function in a control subject known to be free of the nervous system disease, including, for example, by administering a PPA as presently disclosed. For example, these and related embodiments contemplate a method of treating a subject known to have or suspected of having a nervous system disease wherein the nervous system disease may be at least one of psychosis, schizophrenia (in which there is an increase in the level of nerve cells that may be manifest as hyperactive dopamine signaling); epileptic seizures (in which there is an increase in the level of nerve cells that may be manifest as glutamatergic excitotoxicity), ischemic stroke (in which there is an increase in the level of nerve cells that may be manifest as glutamatergic excitotoxicity), and insomnia associated with restless leg syndrome (in which there is an increase in the level of nerve cells that may be manifest as overactive glutamatergic activity).

In certain embodiments there is provided a method of treating, reducing the severity of, or reducing the likelihood of occurrence of disease in a subject known to have or suspected of having a CNS condition associated with CNS pericyte loss or CNS pericyte dysfunction, wherein the nervous system disease is characterized by presence in the subject of cerebrospinal fluid (CSF) that comprises an altered level of one or more cerebrospinal fluid (CSF) components, relative to the level of said CSF component or components in a control subject known to be free of the CNS condition, including, for example, by administering a PPA as presently disclosed.

By way of non-limiting theory, certain PPA identified according to the present disclosure may be CSF components that are produced by CP cells,

such that administering a PPA may prove to be therapeutically beneficial. Representative CSF components are set forth in Figure 3. Additionally or alternatively according to certain herein disclosed embodiments, the PPA comprises an H_2O_2-sensitive, thermostable, hexane-insoluble/ diethyl ether-insoluble/ ethyl acetate-insoluble and water-soluble molecule having a molecular weight of less than 3 kDa that is obtained in a culture medium that has been conditioned by cultured mammalian choroid plexus (CP) cells (*e.g.*, encapsulated CP cells to obtain CPe conditioned medium), wherein the PPA is capable, upon being contacted with CNS pericytes, of decreasing a level of CNS pericyte loss or CNS pericyte dysfunction, relative to the level of CNS pericyte loss or CNS pericyte dysfunction when the PPA is absent. Certain embodiments contemplate, for use as the PPA, a synthetic or artificial mimetic of the H_2O_2-sensitive, thermostable, hexane-/ diethyl ether-/ ethyl acetate-insoluble and water-soluble molecule having a molecular weight of less than 3 kDa that is obtained in a culture medium that has been conditioned by cultured mammalian CP cells (*e.g.*, encapsulated CP cells to obtain CPe conditioned medium), wherein the PPA is capable, upon being contacted with CNS pericytes, of decreasing a level of CNS pericyte loss or CNS pericyte dysfunction, relative to the level of CNS pericyte loss or CNS pericyte dysfunction when the PPA is absent. For example, these and related embodiments contemplate a method of treating a subject known to have or suspected of having a nervous system disease wherein the nervous system disease may be at least one of Alzheimer's disease and diabetes mellitus.

In certain embodiments there is provided a method of treating, reducing the severity of, or reducing the likelihood of occurrence of disease in a subject known to have or suspected of having a CNS condition associated with CNS pericyte loss or CNS pericyte dysfunction, including, for example, by administering a PPA as presently disclosed, wherein the nervous system disease is characterized by presence in the subject of an altered level of at least one choroid plexus function, relative to the level of said choroid plexus

function in a control subject known to be free of the nervous system disease. For example, these and related embodiments contemplate a method of treating a subject known to have or suspected of having a CNS condition that may be Sturge-Weber syndrome, or Klippel-Trenaunay-Weber syndrome, or any of a number of other clinically identifiable congenital nervous system diseases having recognized diagnostic signs and symptoms.

In certain embodiments there is provided a method of treating, reducing the severity of, or reducing the likelihood of occurrence of disease in a subject known to have or suspected of having a CNS condition associated with CNS pericyte loss or CNS pericyte dysfunction, wherein the nervous system disease in the subject is a secondary effect of increased (*e.g.*, in a statistically significant manner) amyloid deposit in the endothelium and smooth muscle cells in the nervous system of the subject, relative to the level of said deposit in a control subject known to be free of the nervous system disease (*e.g.,* Ghiso et al., 2001 *J. Alzheimer's Dis.* 3:65), including, for example, by administering a PPA as presently disclosed. For example, these and related embodiments contemplate a method of treating, reducing the severity of, or reducing the likelihood of occurrence of disease in a subject known to have or suspected of having a CNS condition that may be cerebral amyloid angiopathy, hereditary cerebral hemorrhage with amyloidosis-Icelandic type (HCHWA-I), cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D), meningocerebrovascular and oculoleptomeningeal amyloidosis, gelsolin-related spinal and cerebral amyloid angiopathy, familial amyloidosis-Finnish type (FAF), vascular variant prion cerebral amyloidosis, familial British dementia (FBD), otherwise known as familial cerebral amyloid angiopathy-British type or cerebrovascular amyloidosis-British type, familial Danish dementia, also known as heredopathia ophthalmo-oto-encephalica, familial transthyretin (TTR) amyloidosis, or PrP cerebral amyloid angiopathy (PrP-CAA) (Ghiso et al. 2001 *J Alzheimer's Dis* 3:65).

METHOD OF TREATING

Preferred embodiments contemplate a method of treating, reducing severity of, or reducing likelihood of occurrence of disease in a subject that is a human or non-human mammal known to have or suspected of having or suspected of being at risk for developing a CNS condition associated with CNS pericyte loss or CNS pericyte dysfunction, by administering one or more pericyte protective agents (PPA) as provided herein (*e.g.*, as identified according to the presently disclosed methods and also including mimetics of such PPA as may also be identified according to the presently disclosed methods). Mammals thus may include humans, and also may include domesticated animals such as laboratory animals, livestock and household pets (*e.g.*, rodents, cats, dogs, rabbits and other lagomorphs, swine, cattle, sheep, goats, horses, other ungulates, etc.), and also non-domesticated animals such as wildlife and the like. According to certain herein disclosed embodiments, the PPA comprises an H_2O_2-sensitive, thermostable, hexane-insoluble/ diethyl ether-insoluble/ ethyl acetate-insoluble and water-soluble molecule having a molecular weight of less than 3 kDa that is obtained in a culture medium that has been conditioned by cultured mammalian choroid plexus (CP) cells (*e.g.*, encapsulated CP cells to obtain CPe conditioned medium), wherein the PPA is capable, upon being contacted with CNS pericytes, of decreasing a level of CNS pericyte loss or CNS pericyte dysfunction, relative to the level of CNS pericyte loss or CNS pericyte dysfunction when the PPA is absent.

A "therapeutically effective amount" refers to that amount of a composition or preparation according to the present disclosure which, when administered to a mammal, preferably a human, is sufficient to effect treatment of a CNS condition associated with CNS pericyte loss or CNS pericyte dysfunction in the mammal, preferably a human. The amount of such a composition or preparation, such as one or more pericyte protective agents (PPA) as provided herein (*e.g.*, as identified according to the presently disclosed methods and also including mimetics of such PPA as may also be identified according to the

presently disclosed methods), which constitutes a "therapeutically effective amount" will vary depending on the composition or preparation, the CNS condition associated with CNS pericyte loss or CNS pericyte dysfunction and its severity, and the age of the mammal to be treated, but can be determined routinely by one of ordinary skill in the art having regard to such person's own knowledge and to this disclosure.

In preferred embodiments the PPA may be administered under conditions that permit the PPA to contact CNS pericytes in the subject, according to any of a number of known therapeutic delivery strategies including approaches designed to permeate the BBB. Non-limiting examples of techniques for accessing the BBB as may afford contact to CNS pericytes for such purposes are described in, *e.g.,* Aly et al., 2015 *Expert Opin Drug Deliv* 12:1923; Vangilder et al., 2011 *Pharmacol. Ther.* 130:239; ElAli et al., 2014 *Int J Mol Sci. 15*:6453; Garbayo et al., 2013 *Maturitas* 76:272; Mikitsh et al., 2014 *Perspect Medicin Chem* 6:11; Georgieva et al., 2014 *Pharmaceutics* 6:557; Patel et al., 2007 *Acta Neurochir Suppl* 97(Pt 2):135; Patel et al., 2005 *Ann. Neurol.* 57:298; Kirik et al., 2004 *Nat. Neurosci.* 7:105; Bender et al., 2015 *Neuroscience* 303:569; Tajes et al., 2014 *Mol. Membr. Biol.* 31:152; Abbott, 2013 *J Inherit Metab Dis.* 36:437; Zhang et al., 2016 *Mol. Pharm.* 13:1540; De Rosa et al., 2012 *Curr. Drug Metab.* 13:61; Sweeney et al., 2016 *Nat. Neurosci.* 19:771.

"Treating" or "treatment" refers to therapy to heal, relieve symptoms of and/or correct underlying defects contributing to or causes of the CNS condition associated with CNS pericyte loss or CNS pericyte dysfunction in a mammal, preferably a human, having the disease or disorder of interest (*e.g.*, a neurodegenerative disease), and includes inhibiting (*e.g*, impairing, reducing or preventing, such as decreasing in a statistically significant manner) or repairing (*e.g.*, replacing, supplementing or substituting for) a defective molecular, cellular, and/or tissue component that contributes to the CNS disease, disorder or condition and/or a deleterious process that contributes to the CNS disease,

disorder or condition, to a substantial and statistically significant degree of inhibition or repair (although not necessarily complete), *e.g.*, at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% or greater inhibition or repair relative to appropriate untreated controls; and also includes partially or completely relieving the signs or symptoms resulting from the disease, disorder or condition, *e.g.,* reducing inflammatory lesions associated with disease, restoring one or more normal BBB, CNS vascular, neuronal and/or glial cell structures and/or functions, etc.

It will be appreciated that the practice of the several embodiments of the present invention will employ, unless indicated specifically to the contrary, conventional methods in virology, immunology, microbiology, molecular biology and recombinant DNA techniques that are within the skill of the art, and many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. *See*, *e.g.*, *Current Protocols in Molecular Biology* or *Current Protocols in Immunology*, John Wiley & Sons, New York, N.Y.(2009); Ausubel *et al.*, *Short Protocols in Molecular Biology*, 3rd ed., Wiley & Sons, 1995; Sambrook and Russell, *Molecular Cloning: A Laboratory Manual* (3rd Edition, 2001); Maniatis *et al. Molecular Cloning: A Laboratory Manual* (1982); *DNA Cloning: A Practical Approach*, vol. I & II (D. Glover, ed.); *Oligonucleotide Synthesis* (N. Gait, ed., 1984); *Nucleic Acid Hybridization* (B. Hames & S. Higgins, eds., 1985); *Transcription and Translation* (B. Hames & S. Higgins, eds., 1984); *Animal Cell Culture* (R. Freshney, ed., 1986); Perbal, *A Practical Guide to Molecular Cloning* (1984) and other like references.

Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (*e.g.*, electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. These and related techniques

and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. Unless specific definitions are provided, the nomenclature utilized in connection with, and the laboratory procedures and techniques of, molecular biology, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques may be used for recombinant technology, molecular biological, microbiological, chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

As used in this specification and the appended claims, the singular forms “a,” “an” and “the” include plural references unless the content clearly dictates otherwise. Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers. Each embodiment in this specification is to be applied *mutatis mutandis* to every other embodiment unless expressly stated otherwise.

EQUIVALENTS: While particular steps, elements, embodiments and applications of the present invention have been shown and described herein for purposes of illustration, it will be understood, of course, that the invention is not limited thereto since modifications may be made by persons skilled in the art, particularly in light of the foregoing teachings, without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

The following Examples are presented by way of illustration and not limitation.

EXAMPLES

EXAMPLE 1

SELECTION OF CHOROID PLEXUS (CP) CELL-CONTAINING CAPSULES FOR ELEVATED CEREBROSPINAL FLUID (CSF) PRODUCTION

This example describes selection of CP cell-containing capsules for elevated CSF production using the CSF component VEGF as a representative indicator of CSF production. (See US 2016/0361365 and WO 2016/187067.)

Neonatal porcine choroid plexus tissue was processed and encapsulated in alginate capsules essentially as described in US2009/0047325 and US2009/0214660. Briefly, CP tissue was sterilely dissected from neonatal pig brains, finely chopped with scissors, digested with collagenase and thermolysin, and passed through a 550 µm stainless steel filter, pelleted and gently resuspended to obtain tissue fragments comprising cell clusters of about 50-200 µm in diameter. CP cell clusters were separated from blood cells by unit gravity sedimentation twice for 40 minutes at room temperature. The settled CP cells were resuspended in RPMI medium/2% neonatal porcine serum at a density of approximately 3,000 clusters per mL and cultured in ultra-low attachment flasks for 6-7 days as described, yielding spherical, ovoid and branched CP cell clusters. The cell clusters were incubated in sterile saline solution containing high mannuronic acid containing alginate, droplet-sprayed through a droplet generator into a 109 mM calcium chloride gelation solution, and successively coated with poly-L-ornithine and alginate, to obtain semi-permeable capsules substantially all of which were about 400 µm to about 800 µm in diameter. The capsules were then treated with 55 mM iso-osmolar sodium citrate for 2 minutes in a rolling 50 mL tube. Capsules were maintained in culture medium containing serum and aliquots sampled to confirm cell viability.

The amounts of VEGF secreted per cell were compared in aliquots of equivalent numbers of unselected (random) CP cell-containing capsules and selected CP cell-containing capsules. Selected CP cell-containing capsules were hand-picked, on the basis of direct microscopic observation, for the presence of 200-to-10,000 encapsulated cells per capsule, where the capsules exhibited a smooth exterior surface uninterrupted by protruding cells or cellular processes from the capsule interior or by superficially attached cells or tissue fragments. Culture wells of a 24-well multi-well plate were seeded either with 500 unselected (random) CP cell-containing capsules or 500 selected capsules and cultured at 37°C for 24 hours. Aliquots of supernatant fluids were collected and assayed for VEGF using an ELISA kit (Human VEGF Quantikine™ ELISA, Cat. #DVE00, R & D Systems, Minneapolis, MN) according to the manufacturer's instructions. Aliquots of the cell cultures were also collected for DNA quantification using a Quant iT™ Picogreen dsDNA assay according to the supplier's instructions (Cat. # P7589, Life Technologies, Inc./Thermo Fisher Scientific, Grand Island, NY) to determine the relative number of cells in each well.

Comparative DNA quantification of the culture wells revealed that wells receiving 500 selected capsules (200-10,000 encapsulated cells per capsule) contained three times as much DNA as wells that had received 500 unselected (random) capsules. Supernatant fluids of wells that had received 500 selected capsules contained six times as much VEGF than the supernatants from unselected (random) capsule cultures. When the samples were normalized to DNA content as a reflection of the amount of VEGF secreted on a per cell basis, selected capsules were found to secrete slightly more than twice as much VEGF per µg of DNA than unselected capsules (See US 2016/0361365 and WO 2016/187067). In a representative batch of encapsulated CP cells, greater than 90% of capsules contained viable cells, as visualized by phase-contrast microscopy, nuclear staining, and vital dye exclusion.

EXAMPLE 2

PROTECTION OF CULTURED HUMAN CENTRAL NERVOUS SYSTEM (CNS) PERICYTES BY ENCAPSULATED PORCINE CHOROID PLEXUS (CP) CELL-DERIVED PERICYTE PROTECTIVE AGENT (PPA)

This example describes the protective effects of encapsulated porcine choroid plexus (CP) cell-derived product(s) against pericyte stressor-induced CNS pericyte loss and CNS pericyte dysfunction, using cultured human CNS pericytes.

Methods: Human CNS pericytes were isolated from neurologically normal post-mortem adult human brain tissue through mechanical dissociation and enzymatic digestion as described (Rustenhoven et al., 2015 *Sci Rep*. 5: 12132). Cells were cultured until passage four to eliminate non-proliferative astrocytes, microglia, and endothelial cells.

Porcine choroid plexus (CP) cells were isolated, encapsulated, and maintained in culture as described in Example 1 and in US 2016/0361365 and WO 2016/187067. Human CNS pericytes and encapsulated porcine CP cells (capsules) were cultured in 96-well plates seeded with 5×10^3 human CNS pericytes per well under varying culture conditions as follows: (1) human CNS pericytes in DMEM/F12, (2) human CNS pericytes in 1:1 (v/v) DMEM/F12:CP non-conditioned media, (3) human CNS pericytes in 1:1 (v/v) DMEM/F12:CP conditioned media, or (4) human CNS pericytes in 1:1 (v/v) DMEM/F12:CP capsules.

Initial screens included determination of human CNS pericyte viability, proliferation, inflammatory response, and overall cell numbers. Proliferation was determined by DNA incorporation of the thymidine analogue 5-ethynyl-2'-deoxyuridine (EdU®; 10 μM) for 24 hours and visualization using Click-iT™ chemistry (Invitrogen, Carlsbad, CA) and Hoechst 33258 as a nuclear counterstain. PDGFBB and $TGF\beta_1$ were included as positive and

negative controls for enhancement and reduction of proliferation respectively. Viability was determined in response to 0-1000 μM H_2O_2 to model oxidative stress present in several neurodegenerative diseases using the ReadyProbes™ cell viability imaging kit and an LDH cytotoxicity assay according to the manufacturer's instructions (Roche Diagnostics Corp., Indianapolis, IN). Concentrations between 0-1000 μM H_2O_2 were found to be appropriate for this purpose.

Three separate batches of encapsulated CP cells were screened and exhibited substantially similar characteristics in culture; the pericyte protective effects of conditioned media from these batches were also comparable. Two additional separate preparations of encapsulated CP cells were used to generate conditioned media that also had comparable protective effects on cultured human CNS pericytes. Explants from each batch of encapsulated CP cells were examined for viability using the ReadyProbes™ cell viability imaging kit according to the manufacturer's instructions (Molecular Probes, Eugene, OR) and were found to be >95% viable. Approximately 10-20 CP capsules per well were introduced to cultured pericytes for co-culture experiments.

Experiments were designed to assess the potential protective effects of conditioned media generated by cultured encapsulated CP cells, and of co-culturing with the encapsulated CP cells, on cultured human CNS pericyte survival and inflammatory responses. Human CNS pericytes (5×10^3 cells/well) were cultured for 48 hours, and were then incubated with CP cell-containing capsules or with encapsulated CP cell-conditioned media for 24 hours prior to exposing the pericytes for a further 24 hours to varying concentrations of the stressor H_2O_2 or to the inflammatory mediator IL-1β. Pericyte viability was determined in response to 0-1000 μM H_2O_2 to model oxidative stress using the ReadyProbes™ cell viability imaging kit (Roche).

The ability of CP cells, encapsulated CP clusters (capsules) or CP-conditioned medium derived from free and/or encapsulated CP clusters to

modify basal or IL-1β-induced inflammatory responses was determined by immunocytochemical analysis for altered (*e.g.,* increased or decreased in a statistically significant manner relative to appropriate controls) levels of two common pericyte inflammatory markers, the adhesion molecule intercellular adhesion molecule-1 (ICAM-1) and the chemokine monocyte chemoattractant protein-1 (MCP-1), in response to 0-10 ng/mL IL-1β.

All imaging was performed using the ImageXpress™ automated fluorescence microscope (Molecular Devices, Sunnyvale, CA) and image analysis was performed using MetaXpress™ software (Molecular Devices), both according to the manufacturer's instructions.

Results: Incubation of cultured human CNS pericytes for 24 hours with encapsulated CP cells or CP capsule-conditioned media prior to stressing the pericytes by H_2O_2 addition revealed a remarkable increase in pericyte viability compared to the viability of control pericytes preincubated with non-conditioned media. The ability of CP capsules to attenuate pericyte death was investigated in response to 0-1000 μM H_2O_2 in order to model oxidative stress. Pericytes were plated at 5,000 cells/well and maintained in culture for two days, after which a full media change was performed by which four treatment conditions were compared: (i) DMEM/F12, (ii) 1:1 ratio of DMEM/F12:CP non-conditioned media, (iii) 1:1 ratio of DMEM/F12:CP conditioned media, or (iv) 1:1 ratio of DMEM/F12:CP capsules.

For treatment groups receiving encapsulated CP cells, approximately 10 capsules were added per well. Pericytes were cultured for 24 hours before addition of 0-1000 μM H_2O_2 for a further 24 hours. Figure 1(1a/1b) shows determination of the percentage of dead cells (Fig. 1a) and total cell number (Fig. 1b) for treatment groups using three independently produced batches ("(1)", "(2)", "(3)") of encapsulated CP cells and CP capsule-conditioned media. Similar effects were seen when two additional independently generated batches ("(4)", "(5)") of CP capsule-conditioned media were tested, as shown in Figure 2(2a/2b) (Fig. 2a, percentage of dead cells; Fig. 2b, total cell number).

This pericyte-protective effect was achieved through the prevention of cell membrane rupture, as visualized by vital dye exclusion, and could not be explained simply by an alteration in the number of pericytes. This protective effect was observed using three independently produced preparations of encapsulated CP cells and conditioned media (Fig. 1a, 1b) and two additional independently generated preparations of encapsulated CP cell-conditioned media (Fig. 2a, 2b). Pericyte protective effects were manifested as reductions in H_2O_2-induced toxicity that, by way of non-limiting theory, correlated with increased time in culture of the encapsulated CP cells (*e.g.*, CP capsule age) producing the conditioned media. Exemplary encapsulated CP cells were produced as described above and maintained in culture at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 or more days prior to use as sources of conditioned medium containing pericyte protective agents and/or in pericyte co-cultures.

Encapsulated CP cells, or encapsulated CP cell-conditioned media, were also tested for their effects on the ability of cultured human CNS pericytes to exhibit markers of an inflammatory response to the pro-inflammatory cytokine IL-1β. Human CNS pericytes co-cultured with encapsulated CP cells or encapsulated CP cell-conditioned media displayed slightly reduced induction of the inflammatory markers MCP-1 and ICAM-1 in response to low concentrations of IL-1β (0.01-0.1 ng/mL) (not shown). Co-culturing of pericytes with any of the five preparations described in this Example of encapsulated CP cells or encapsulated CP cell-conditioned media did not significantly affect the maximal pericyte inflammatory responses (MCP-1, ICAM-1) to higher concentrations (1 – 10 ng/mL) of IL-1β.

EXAMPLE 3

PHYSICOCHEMICAL PROPERTIES OF PERICYTE PROTECTIVE AGENT (PPA) PRESENT IN CONDITIONED MEDIUM FROM ENCAPSULATED PORCINE CHOROID PLEXUS (CP) CELLS

This example describes characterization of the functional and physicochemical properties of a pericyte protective agent (PPA) that was present in tissue culture medium that was conditioned by encapsulated choroid plexus (CP) cells (CPe). Materials and methods for the preparation and maintenance of human CNS pericytes, and of encapsulated porcine CP cells and conditioned culture media produced therefrom, were essentially as described above in Examples 1 and 2. Assays of the protective effects against H_2O_2 oxidative stress on pericyte viability, pericyte proliferation, and pericyte function that were conferred by the PPA present in encapsulated CP cell-conditioned media (CPe) were also essentially as described above in Examples 1 and 2.

Briefly, human CNS pericytes were isolated from neurologically normal post-mortem adult human brain tissue and tested for susceptibility to H_2O_2-induced cell death as described above. H_2O_2 concentrations were quantified using the Pierce™ Quantitative Peroxide assay kit (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions, with absorbance measurements (A_{595}) taken on a FluoStar™ plate reader (Thermo Fisher). Titration of H_2O_2 was performed in each assay to control for variability among cultures in the effects of this oxidative stressor on pericyte viability, and assays in which H_2O_2-induced cell death in the absence of any PPA was lower than 75% were excluded.

Encapsulated choroid plexus cell-conditioned medium (CPe), and a panel of known compounds as candidate PPAs, were tested for their abilities to protect pericytes from H_2O_2-induced cell death. CPe exhibited PPA activity

by protecting pericytes against H_2O_2-induced cell death (Fig. 4), an effect that was shown to be concentration-dependent, by serial dilution of the CPe conditioned medium with fresh medium. Among known compounds that were tested for potential PPA activity, detectable protection of pericytes from H_2O_2-induced cell death was conferred by nicotinamide (200 μM – 10 mM) and VEGF (31.3 pg/mL, 264.1 pg/mL, and 689.6 pg/mL respectively, for VEGF-K1, VEGF-K2, and VEGF-K3) but not by ascorbic acid (75-200 μM) or α-tocopherol (25-250 μM); only weak protection was observed when IGF-1 or IGF-2 (10-100 ng/mL) was used as a candidate PPA. Similar levels of PPA activity were detected in conditioned media from encapsulated CP cells (CPe) and in conditioned media from non-encapsulated CP cells, as assessed using the alamarBlue® (resazurin-to-resorufin redox conversion) cell activity/ viability assay (ThermoFisher Scientific, Waltham, MA) according to the manufacturer's recommendations. Comparable levels of PPA activity were detected in CPe conditioned medium that was obtained after encapsulated CP cells were maintained in culture over periods of from 30 to over 150 days.

Physicochemical properties of the CPe-conditioned medium components responsible for PPA activity were assessed.

PPA Redox Sensitivity. The PPA activity of CPe-conditioned medium (CPe) was tested for oxidation state sensitivity by exposure to hydrogen peroxide (H_2O_2). CPe was pretreated with 450 μM H_2O_2 for 24 hours at room temperature prior to incubating pericytes with the H_2O_2-pretreated CPe (1:1 v/v in DMEM) for 24 hours at 37°C in advance of exposing the pericytes to the H_2O_2 stressor (450 μM for 24 h at 37°C) and assessing their viability and functional state by, respectively, the ReadyProbes® vital dye exclusion viability assay and the alamarBlue® redox state assay for cellular activity. Control groups of pericytes received CPe that had not been pre-treated with H_2O_2. CPe PPA activity was observed to be compromised (*i.e.,* decreased in a statistically significant manner relative to appropriate controls) by the H_2O_2 pre-treatment,

suggesting that the CPe component responsible for PPA activity was a redox-sensitive compound.

PPA Molecular Weight. CPe-conditioned medium having PPA activity was size-fractionated by ultrafiltration, initially to obtain fractions of CPe components having molecular weights above 10 kDa and below 10 kDa, and subsequently (see below) to obtain fractions of CPe components having molecular weights above 3 kDa and below 3 kDa.

Briefly, Millipore Amicon® Ultra (MilliporeSigma, Burlington, MA) centrifugal filter units containing regenerated cellulose with 10 kDa or 3 kDa exclusion membranes were loaded with MilliQ® (MilliporeSigma) purified water (~ 3 mL each), balanced, then centrifuged at 4000 rpm (RCF = 2700 × g) for 10 minutes, which resulted in approximately 1.2 mL of water passing through the membranes of each filter unit. After this pre-treatment, the water was discarded and the filter units were each loaded with approximately 3.7 mL of media, being an equally divided single batch (typically approximately 7.5 mL in total), then centrifuged at 4000 rpm (RCF = 2700× g) for 10 minutes, which resulted in approximately 1.2 mL of media passing through the membranes of each filter unit. The filter units were inspected before being further centrifuged at 4000 rpm (RCF = 2700 × g) for an additional 10 minutes, resulting in a volume of approximately 2.5 mL having passed through the exclusion membranes of each unit. The low MW fractions were combined (approximately 5 mL total) and then diluted with MilliQ® water to a total volume of approximately 15 mL.

These fractions were tested for PPA activity as described above, by preincubating them with cultured human CNS pericytes prior to challenging the pericytes with the H_2O_2 stressor and assessing the cells for cell viability and functional state by, respectively, the ReadyProbes® vital dye exclusion viability assay and the alamarBlue® redox state assay for cellular activity. No detectable PPA activity was present in the CPe fraction containing components having molecular weight greater than 10 kDa, while the fraction of components

having molecular weight less than 10 kDa was observed to contain potent PPA activity similar to that found in unfractionated CPe.

To further define the molecular weight range of the CPe component responsible for PPA activity, CPe conditioned medium was size fractionated as described above using an ultrafiltration membrane having a 3 kDa exclusion limit, and the resulting fractions were tested for PPA activity. Substantially no detectable PPA activity was present in the CPe fraction containing components having molecular weight greater than 3 kDa, while the fraction of components having molecular weight less than 3 kDa was observed to contain potent PPA activity similar to that found in unfractionated CPe (Fig. 4). These results suggested that the CPe component responsible for PPA activity had a molecular weight of less than 3 kDa.

PPA Solubility. The solubility properties of the low molecular weight (< 3 kDa) CPe component associated with PPA activitiy were investigated by differential extraction of CPe with organic solvents of progressively increasing polarity (hexane, diethyl ether, and ethyl acetate), and the PPA activity in each extract was compared to the PPA activity retained by the aqueous (water-soluble) fraction.

The combined low molecular weight (< 3 kDa) fractions obtained as described above following recovery from ultrafiltration using a 3 kDa MWCO membrane and dilution with MilliQ®-purified water (to a total volume of approximately 15 mL) were extracted with hexanes (3 × 6 mL) with protracted swirling. Organic and aqueous phases were permitted to separate and the organic fractions were then combined, washed with MilliQ® purified water (1 × 2 mL), and concentrated *in vacuo* prior to lyophilization to obtain a first organic extract residue (hexane extractable material). The remaining aqueous fraction was then extracted with diethyl ether following the same protocol as was used for the hexane extraction described above, prior to lyophilization to obtain a second organic extract residue comprising diethyl ether extractable material. Following diethyl ether extraction, the remaining aqueous fraction was extracted

with ethyl acetate, again according to the same protocol as described above for hexane extraction, prior to lyophilization to obtain a third organic extract residue comprising ethyl acetate extractable material. The remaining aqueous fraction was then lyophilized and extracted with anhydrous methanol by Soxhlet extraction for two hours. The methanol-extracted material from the aqueous fraction so obtained was concentrated *in vacuo* and then lyophilized, and any remaining compounds in the thimble of the Soxhlet extractor were dissolved in MilliQ®-purified water, and then lyophilized.

The residues resulting from each extraction and lyophilization were then reconstituted with MilliQ® water to the initial media volume in order to maintain consistent concentration of the active species. These reconstituted extracts were then tested for PPA activity in the *in vitro* H_2O_2-stressed pericyte system as described above, by preincubating them with cultured human CNS pericytes prior to challenging the pericytes with the H_2O_2 stressor and assessing the cells for cell viability and functional state by, respectively, the ReadyProbes® vital dye exclusion viability assay and the alamarBlue® redox state assay for cellular activity.

Substantially no detectable PPA activity was present in the organic solvent-extractable CPe fractions containing CPe-derived components that were soluble in hexane, diethyl ether, or ethyl acetate, as summarized for pericyte cellular viability data in Fig. 4. The aqueous fraction of CPe, however, containing persistent water-soluble CPe components that had not partitioned into the organic solvents, was observed to contain potent PPA activity similar to that found in unfractionated CPe (Fig. 4). These results suggested that the CPe component responsible for PPA activity was relatively hydrophilic. In subsequent experiments, diluted aqueous low molecular weight (< 3 kDa) fractions of CPe were prepared as described above and initially extracted with ethyl acetate, prior to lyophilization of the aqueous fraction and Soxhlet extraction (as also described above). PPA activity levels in the aqueous fraction following organic extraction only with ethyl acetate prior to lyophilization

and Soxhlet extraction were observed that were comparable to the PPA activity levels detected in the aqueous fraction that had been serially extracted with hexane, diethyl ether, and then ethyl acetate prior to lyophilization and Soxhlet extraction.

PPA Temperature Sensitivity. The thermostability properties of the low molecular weight (< 3 kDa) CPe component associated with PPA activity were investigated by examining the effects of heat treatment on PPA activity in the low molecular weight (< 3 kDa) fractions prepared by ultrafiltration of CPe and dilution with MilliQ® water as described above. A single batch (15 mL) of diluted CPe (< 3 kDa fraction) was divided into two aliquots of approximately 7.5 mL each. One aliquot was heated to boiling (100 °C) under a cooling condenser to prevent solvent loss and refluxed by maintaining at the boiling point for 2.5 hours with vigorous stirring, while the control aliquot was maintained at room temperature. Further fractionation by differential solvent extraction as described above was performed on both the control ("Fresh") and heat-treated ("Reflux") fractions.

Cultured human pericytes were incubated for 24 hours with either heat-treated CPe ("Reflux") or control CPe ("Fresh") in advance of exposing the pericytes to the H_2O_2 stressor and assessing their viability and functional state by, respectively, the ReadyProbes® vital dye exclusion viability assay and the alamarBlue® redox state assay for cellular activity. As shown in Fig. 4 ("Thermostable"), the two CPe preparations were substantially comparable in their ability to protect pericytes from the deleterious effects on cell viability of oxidative stress by H_2O_2. Similar retention of comparable PPA activity between "fresh" and "reflux" samples was exhibited by the low molecular weight (< 3 kDa) PPA-containing fraction of CPe prepared as described above, and by the water soluble PPA component of the < 3 kDa fraction following the hexane/ diethyl ether/ ethyl acetate extractions described above. These results suggested that the CPe component responsible for PPA activity was largely

unaffected by heat treatment and was therefore regarded as exhibiting thermostability.

The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications and publications to provide yet further embodiments.

These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

What is claimed is:

1. A screening method for identifying a pericyte protective agent (PPA) that protects a central nervous system (CNS) pericyte from a pericyte stressor, wherein said pericyte stressor is capable of inducing at least one of CNS pericyte loss and CNS pericyte dysfunction, the method comprising:

(a) contacting, simultaneously or sequentially and in any order, (i) a cultured human CNS pericyte with (ii) the pericyte stressor that is capable of inducing at least one of CNS pericyte loss and CNS pericyte dysfunction, and (iii) a candidate pericyte protective agent (PPA), under conditions and for a time sufficient to induce detectable CNS pericyte loss or detectable CNS pericyte dysfunction when the PPA is absent, thereby to obtain a human CNS pericyte test culture; and

(b) detecting, in the human CNS pericyte test culture of (a), a level of at least one of CNS pericyte loss and CNS pericyte dysfunction that is decreased relative to the level that is detected when the PPA is absent, and thereby identifying the candidate PPA as a pericyte protective agent (PPA).

2. A screening method for identifying a pericyte protective agent (PPA) that protects a peripheral nervous system (PNS) pericyte from a pericyte stressor, wherein said pericyte stressor is capable of inducing at least one of PNS pericyte loss and PNS pericyte dysfunction, the method comprising:

(a) contacting, simultaneously or sequentially and in any order, (i) a cultured human PNS pericyte with (ii) the pericyte stressor that is capable of inducing at least one of PNS pericyte loss and PNS pericyte dysfunction, and (iii) a candidate pericyte protective agent (PPA), under conditions and for a time sufficient to induce detectable PNS pericyte loss or

detectable PNS pericyte dysfunction when the PPA is absent, thereby to obtain a human PNS pericyte test culture; and

(b) detecting, in the human PNS pericyte test culture of (a), a level of at least one of PNS pericyte loss and PNS pericyte dysfunction that is decreased relative to the level that is detected when the PPA is absent, and thereby identifying the candidate PPA as a pericyte protective agent (PPA).

3. The method of claim 1 or claim 2 wherein the candidate PPA is produced by mammalian choroid plexus (CP) cells.

4. The method of claim 3 wherein the candidate PPA is present in a culture medium that has been conditioned by the mammalian CP cells.

5. The method of claim 4 wherein the culture medium has been separated from the mammalian CP cells and has been conditioned by at least one of:

(i) mammalian CP cells that are present in one or more semi-permeable biocompatible capsules in which are encapsulated choroid plexus (CP) tissue fragments that are obtained by either or both of mechanical and enzymatic dissociation of mammalian choroid plexus tissue to obtain CP cell clusters that are about 50 μm to at least about 200 μm in diameter and that comprise CP epithelial cells;

(ii) cultured non-encapsulated CP cells obtained from mammalian choroid plexus tissue; and

(iii) choroid plexus (CP) cells that are obtained by culturing a population of mammalian pluripotent cells under conditions and for a time sufficient to obtain a plurality of *in vitro* differentiated choroid plexus (CP) cells.

6. The method of claim 4 wherein the mammalian CP cells are present in one or more semi-permeable biocompatible capsules in which are encapsulated choroid plexus (CP) tissue fragments that are obtained by either or both of mechanical and enzymatic dissociation of mammalian choroid plexus tissue to obtain CP cell clusters that are about 50 μm to at least about 200 μm in diameter and that comprise CP epithelial cells.

7. The method of claim 6 wherein substantially all of said capsules are about 400 μm to about 800 μm in diameter and have about 200 to about 10,000 CP cells per capsule.

8. The method of claim 3 wherein the mammalian CP cells are present in one or more semi-permeable biocompatible capsules in which are encapsulated *in vitro* differentiated choroid plexus (CP) cells that are obtained by culturing a population of mammalian pluripotent cells under conditions and for a time sufficient to obtain a plurality of *in vitro* differentiated choroid plexus (CP) cells, substantially all of said capsules being about 400 μm to about 800 μm in diameter and having about 200 to about 10,000 CP cells per capsule.

9. The method of claim 3 wherein the mammalian choroid plexus cells are from mammalian choroid plexus tissue of a mammal that is allogeneic or xenogeneic relative to the cultured human CNS pericyte or PNS pericyte.

10. The method of claim 9 wherein the mammalian choroid plexus tissue comprises human, porcine, ovine, bovine, caprine, or non-human primate choroid plexus cells.

11. The method of claim 10 wherein the porcine choroid plexus cells are cultured from a tissue that comprises fetal or neonatal choroid plexus tissue.

12. The method of claim 10 wherein the mammalian choroid plexus tissue is substantially free of human pathogens.

13. The method of claim 12 wherein the choroid plexus tissue is substantially free of human-tropic transmissible porcine endogenous retroviruses.

14. The method of claim 12 wherein at least one of: (i) the choroid plexus tissue is substantially incapable of producing infectious human-tropic porcine endogenous retroviruses (PERVs), or (ii) the choroid plexus tissue is obtained from an animal that lacks PERV genes.

15. The method of claim 14 wherein the choroid plexus tissue is obtained from an animal that lacks a PERV-C *env* gene which is capable of recombination with a PERV-A *env* gene.

16. The method of claim 15 wherein the animal that lacks a PERV-C *env* gene which is capable of recombination with a PERV-A *env* gene has been genetically engineered to lack any or all PERV genes.

17. The method of claim 16 wherein the animal that has been genetically engineered to lack any or all PERV genes is produced by Clustered Regularly-Interspaced Short Palindromic Repeats (CRISPR)-Cas9 editing.

18. The method of claim 8 wherein either one or both of:

(i) the population of mammalian pluripotent cells is obtained from a source that is selected from embryonic cells, umbilical cord

cells, placental cells, neural crest progenitors, adult tissue stem cells, and somatic tissue cells; and

(ii) the population of mammalian pluripotent cells is cultured in a culture medium that comprises one or more *in vitro* CP differentiation agents selected from a bone morphogenic protein (BMP) or a BMP signaling pathway agonist, a transforming growth factor-beta (TGF-β) superfamily member or a TGF-β signaling pathway agonist, a nodal protein or a nodal signaling pathway agonist, a mammalian growth and differentiation factor (GDF) or a GDF signaling pathway agonist, a Wnt protein ligand or a Wnt signaling pathway agonist, a fibroblast growth factor (FGF) or an FGF signaling pathway agonist, and sonic hedgehog (Shh) or a Shh signaling pathway agonist, under conditions and for a time sufficient to obtain said plurality of *in vitro* differentiated choroid plexus (CP) cells.

19. The method of claim 18 wherein the Wnt signaling pathway agonist is selected from WAY-316606 (SFRP inhibitor), IQ1 (PP2A activator), QS11 (ARFGAP1 activator), 2-amino-4-[3,4-(methylenedioxy) benzyl-amino]-6-(3-methoxyphenyl) pyrimidine, Norrin, R-spondin-1, R-spondin-2, R-spondin-3, or R-spondin-4, lithium chloride, lithium carbonate, lithium citrate, lithium orotate, lithium bromide, lithium fluoride, lithium iodide, lithium acetate, lithium hydroxide, lithium aluminum hydride, lithium perchlorate, lithium nitrate, lithium diisopropylamide, lithium borohydride, lithium oxide, lithium sulfate, lithium hexafluorophosphate, lithium tetroxide, lithium sulfide, lithium hydride, lithium amide, lithium lactate, lithium tetrafluoroborate, lithium dimethylamide, lithium phosphate, lithium peroxide, lithium manganese oxide, lithium methoxide, lithium metaborate, lithium stearate, or another lithium salt that comprises cationic lithium.

20. The method of claim 1 or 2 wherein the pericyte stressor comprises one or more agents selected from hydrogen peroxide, nitric oxide, tert-butylhydroperoxide, heavily-oxidized glycated LDL, and a pro-apoptotic agent.

21. The method of claim 1 or claim 2 wherein the level of pericyte loss comprises a level of one or more of pericyte cell death, pericyte apoptosis, pericyte necrosis, and pericyte autophagy.

22. The method of claim 1 or claim 2 wherein the level of pericyte dysfunction comprises a level of one or more of reactive oxygen species (ROS) production, reactive nitrogen species (RNS) production, matrix metalloproteinase 2 (MMP2) production, matrix metalloproteinase 9 (MMP9) production, angiopoietin 1 production, fibronectin 1 production, platelet derived growth factor receptor beta (PDGFRβ) expression, connexin 43 expression, NG2 expression, and IL-17R(A/C) expression.

23. The method of claim 1 or claim 2 wherein the candidate PPA is a cerebrospinal fluid (CSF) component that is produced by choroid plexus (CP) cells.

24. A method for treating, reducing severity of, or reducing likelihood of occurrence of a central nervous system (CNS) condition associated with CNS pericyte loss or CNS pericyte dysfunction in a subject, comprising:

administering to the subject a pericyte protective agent (PPA) produced by a choroid plexus (CP) composition,

under conditions that permit the PPA to contact CNS pericytes in the subject to decrease a level of CNS pericyte loss or CNS pericyte dysfunction in the subject relative to the level of CNS pericyte loss or CNS pericyte dysfunction in the subject when the PPA is absent, and thereby treating, reducing the severity of, or reducing the likelihood of occurrence of the central nervous system (CNS) condition associated with CNS pericyte loss or CNS pericyte dysfunction.

25. The method of claim 24 wherein the central nervous system (CNS) condition associated with CNS pericyte loss or CNS pericyte dysfunction is selected from (a) a neurodegenerative disease that is characterized by death of neurons, and (b) a nervous system disease that is selected from Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS, also known as motor neurone disease), ataxia-telangiectasia, progressive bulbar palsy, progressive muscular atrophy, dementia with Lewy bodies, multiple system atrophy, spinocerebellar ataxia type 1 (SCA 1), a retinal degenerative disease, or an age-related neurodegenerative disorder.

26. The method of claim 24 wherein the central nervous system (CNS) condition associated with CNS pericyte loss or CNS pericyte dysfunction is selected from (a) a disease that is characterized by a decrease in a level of at least one nerve cell function, relative to the level of said nerve cell function in a control subject known to be free of the nervous system disease, and (b) the disease of (a) that is selected from Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis, and depression.

27. The method of claim 24 wherein the central nervous system (CNS) condition associated with CNS pericyte loss or CNS pericyte dysfunction is selected from (a) a disease that is characterized by an increase in a level of at least one nerve cell function, relative to the level of said nerve cell function in a control subject known to be free of the nervous system disease, and (b) the disease of (a) that is selected from psychosis, schizophrenia, epileptic seizures, ischemic stroke, and insomnia associated with restless leg syndrome.

28. The method of claim 24 wherein the central nervous system (CNS) condition associated with CNS pericyte loss or CNS pericyte

dysfunction is selected from (a) a disease that is characterized by presence in the subject of cerebrospinal fluid (CSF) that comprises an altered level of one or more cerebrospinal fluid (CSF) components, relative to the level of said CSF component or components in a control subject known to be free of the nervous system disease, and (b) the disease of (a) that is selected from Alzheimer's disease and diabetes mellitus.

29. The method of claim 24 wherein the central nervous system (CNS) condition associated with CNS pericyte loss or CNS pericyte dysfunction is selected from:

(a) a disease that is characterized by presence in the subject of an altered level of at least one choroid plexus function, relative to the level of said choroid plexus function in a control subject known to be free of the nervous system disease,

(b) the disease of (a) that is selected from Sturge-Weber syndrome and Klippel-Trenaunay-Weber syndrome,

(c) a disease that is characterized by an increase in a level of abnormally folded protein deposits in brain tissue of the subject, relative to the level of abnormally folded protein deposits in a control subject known to be free of the nervous system disease, and

(d) the disease of (c) that is selected from cerebral amyloid angiopathy, hereditary cerebral hemorrhage with amyloidosis-Icelandic type (HCHWA-I), cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D), meningocerebrovascular and oculoleptomeningeal amyloidosis, gelsolin-related spinal and cerebral amyloid angiopathy, familial amyloidosis-Finnish type (FAF), vascular variant prion cerebral amyloidosis, familial British dementia (FBD) (also known as familial cerebral amyloid angiopathy-British type or cerebrovascular amyloidosis-British type), familial Danish dementia (also known as heredopathia ophthalmo-oto-encephalica), familial transthyretin (TTR) amyloidosis, and PrP cerebral amyloid angiopathy (PrP-CAA); and

(e) a disease that is caused by blood brain barrier (BBB) dysfunction.

30. The method of claim 24 wherein the central nervous system (CNS) condition associated with CNS pericyte loss or CNS pericyte dysfunction is at least one of (i) a neurodegenerative disease that is characterized by death of CNS neurons, and (ii) a CNS disease characterized by a decrease in a level of at least one CNS nerve cell function, relative to the level of said CNS nerve cell function in a control subject known to be free of the CNS disease, and iii) a CNS disease characterized by an increase in a level of at least one CNS nerve cell function, relative to the level of said CNS nerve cell function in a control subject known to be free of the CNS disease, wherein said CNS neurons and CNS nerve cell are present in at least one of brain, spinal cord, retina, optic nerve, cranial nerve, olfactory nerve or olfactory epithelium.

31. The method of claim 24 wherein the central nervous system (CNS) condition associated with CNS pericyte loss or CNS pericyte dysfunction is one of Parkinson's disease, Alzheimer's disease, and Huntington's disease.

32. The method of claim 25 wherein the retinal degenerative disease is selected from macular degeneration, diabetic retinopathy, and retinitis pigmentosa.

33. A method for treating, reducing severity of, or reducing likelihood of occurrence of a peripheral nervous system (PNS) condition associated with PNS pericyte loss or PNS pericyte dysfunction in a subject, comprising:

administering to the subject a pericyte protective agent (PPA) produced by a choroid plexus (CP) composition,

under conditions that permit the PPA to contact PNS pericytes in the subject to decrease a level of PNS pericyte loss or PNS pericyte dysfunction in the subject relative to the level of PNS pericyte loss or PNS

pericyte dysfunction in the subject when the PPA is absent, and thereby treating, reducing the severity of, or reducing the likelihood of occurrence of the peripheral nervous system (PNS) condition associated with PNS pericyte loss or PNS pericyte dysfunction.

34. The method of claim 33 wherein the condition associated with PNS pericyte loss or PNS pericyte dysfunction is at least one of (i) a neurodegenerative disease that is characterized by death of PNS neurons, and (ii) a PNS disease characterized by a decrease in a level of at least one PNS nerve cell function, relative to the level of said PNS nerve cell function in a control subject known to be free of the PNS disease, and iii) a PNS disease characterized by an increase in a level of at least one PNS nerve cell function, relative to the level of said PNS nerve cell function in a control subject known to be free of the PNS disease, wherein said PNS neurons and PNS nerve cell are present in at least one of a peripheral ganglion or a peripheral nerve.

35. A screening method for identifying a pericyte protective agent (PPA) that protects a central nervous system (CNS) pericyte from a pericyte stressor, wherein said pericyte stressor is capable of inducing at least one of CNS pericyte loss and CNS pericyte dysfunction, the method comprising:

(a) contacting, simultaneously or sequentially and in any order,

(i) a cultured human CNS pericyte,

(ii) the pericyte stressor that is capable of inducing at least one of CNS pericyte loss and CNS pericyte dysfunction,

(iii) a conditioned culture medium that has been conditioned by mammalian choroid plexus (CP) cells and that contains a pericyte protective agent (PPA) produced by said CP cells, or an isolated fraction of said conditioned culture medium that contains the PPA, which PPA is capable of decreasing in the cultured human CNS pericyte a level of at least

one of CNS pericyte loss and CNS pericyte dysfunction, relative to the level that is detected when the PPA is absent, and

(iv) one or a plurality of antibodies that have been generated against a portion of the culture medium that has been conditioned by mammalian choroid plexus (CP) cells and which contains the pericyte protective agent (PPA) produced by said CP cells,

under conditions and for a time sufficient to induce detectable CNS pericyte loss or detectable CNS pericyte dysfunction when the PPA is absent, thereby to obtain a human CNS pericyte test culture;

(b) detecting, in the human CNS pericyte test culture of (a), a level of at least one of CNS pericyte loss and CNS pericyte dysfunction that is decreased to a lesser degree relative to the level that is detected when the one or plurality of antibodies is absent, thereby indicating that the one or plurality of antibodies is capable of neutralizing the PPA; and

(c) isolating the PPA by binding to at least one of the antibodies that is capable of neutralizing the PPA.

36. The method of claim 35 wherein isolating the PPA comprises isolating an immune complex that comprises at least one of the antibodies that is capable of neutralizing the PPA and the PPA.

37. The method of claim 36 which further comprises separating the at least one antibody that is capable of neutralizing the PPA from the PPA and structurally characterizing the PPA.

38. The method of claim 35 wherein the culture medium has been separated from the mammalian CP cells and has been conditioned by at least one of:

(i) mammalian CP cells that are present in one or more semi-permeable biocompatible capsules in which are encapsulated choroid plexus (CP) tissue fragments that are obtained by either or both of mechanical

and enzymatic dissociation of mammalian choroid plexus tissue to obtain CP cell clusters that are about 50 µm to at least about 200 µm in diameter and that comprise CP epithelial cells;

(ii) cultured non-encapsulated CP cells obtained from mammalian choroid plexus tissue; and

(iii) choroid plexus (CP) cells that are obtained by culturing a population of mammalian pluripotent cells under conditions and for a time sufficient to obtain a plurality of *in vitro* differentiated choroid plexus (CP) cells.

39. The method of claim 35 wherein the mammalian CP cells are present in one or more semi-permeable biocompatible capsules in which are encapsulated choroid plexus (CP) tissue fragments that are obtained by either or both of mechanical and enzymatic dissociation of mammalian choroid plexus tissue to obtain CP cell clusters that are about 50 µm to at least about 200 µm in diameter and that comprise CP epithelial cells.

40. The method of claim 39 wherein substantially all of said capsules are about 400 µm to about 800 µm in diameter and have about 200 to about 10,000 CP cells per capsule.

41. The method of claim 35 wherein the mammalian CP cells are present in one or more semi-permeable biocompatible capsules in which are encapsulated *in vitro* differentiated choroid plexus (CP) cells that are obtained by culturing a population of mammalian pluripotent cells under conditions and for a time sufficient to obtain a plurality of *in vitro* differentiated choroid plexus (CP) cells, substantially all of said capsules being about 400 µm to about 800 µm in diameter and having about 200 to about 10,000 CP cells per capsule.

42. The method of claim 35 wherein the mammalian choroid plexus cells are from mammalian choroid plexus tissue of a mammal that is allogeneic or xenogeneic relative to the cultured human CNS pericyte.

43. The method of claim 42 wherein the mammalian choroid plexus tissue comprises human, porcine, ovine, bovine, caprine, or non-human primate choroid plexus cells.

44. The method of claim 43 wherein the porcine choroid plexus cells are cultured from a tissue that comprises fetal or neonatal choroid plexus tissue.

45. The method of claim 43 wherein the mammalian choroid plexus tissue is substantially free of human pathogens.

46. The method of claim 43 wherein the choroid plexus tissue is substantially free of human-tropic transmissible porcine endogenous retroviruses.

47. The method of claim 43 wherein at least one of: (i) the choroid plexus tissue is substantially incapable of producing infectious human-tropic porcine endogenous retroviruses (PERVs), or (ii) the choroid plexus tissue is obtained from an animal that lacks PERV genes.

48. The method of claim 47 wherein the choroid plexus tissue is obtained from an animal that lacks a PERV-C *env* gene which is capable of recombination with a PERV-A *env* gene.

49. The method of claim 48 wherein the animal that lacks a PERV-C *env* gene which is capable of recombination with a PERV-A *env* gene has been genetically engineered to lack any or all PERV genes.

50. The method of claim 49 wherein the animal that has been genetically engineered to lack any or all PERV genes is produced by Clustered Regularly-Interspaced Short Palindromic Repeats (CRISPR)-Cas9 editing.

51. The method of claim 38 wherein either one or both of:

(i) the population of mammalian pluripotent cells is obtained from a source that is selected from embryonic cells, umbilical cord cells, placental cells, neural crest progenitors, adult tissue stem cells, and somatic tissue cells; and

(ii) the population of mammalian pluripotent cells is cultured in a culture medium that comprises one or more *in vitro* CP differentiation agents selected from a bone morphogenic protein (BMP) or a BMP signaling pathway agonist, a transforming growth factor-beta (TGF-β) superfamily member or a TGF-β signaling pathway agonist, a nodal protein or a nodal signaling pathway agonist, a mammalian growth and differentiation factor (GDF) or a GDF signaling pathway agonist, a Wnt protein ligand or a Wnt signaling pathway agonist, a fibroblast growth factor (FGF) or an FGF signaling pathway agonist, and sonic hedgehog (Shh) or a Shh signaling pathway agonist, under conditions and for a time sufficient to obtain said plurality of *in vitro* differentiated choroid plexus (CP) cells.

52. The method of claim 51 wherein the Wnt signaling pathway agonist is selected from WAY-316606 (SFRP inhibitor), IQ1 (PP2A activator), QS11 (ARFGAP1 activator), 2-amino-4-[3,4-(methylenedioxy) benzyl-amino]-6-(3-methoxyphenyl) pyrimidine, Norrin, R-spondin-1, R-spondin-2, R-spondin-3, or R-spondin-4, lithium chloride, lithium carbonate, lithium citrate, lithium orotate, lithium bromide, lithium fluoride, lithium iodide, lithium acetate, lithium hydroxide, lithium aluminum hydride, lithium perchlorate, lithium nitrate, lithium diisopropylamide, lithium borohydride, lithium oxide, lithium sulfate, lithium hexafluorophosphate, lithium tetroxide, lithium sulfide, lithium hydride, lithium amide, lithium lactate, lithium tetrafluoroborate, lithium dimethylamide, lithium phosphate, lithium peroxide, lithium manganese oxide, lithium methoxide,

lithium metaborate, lithium stearate, or another lithium salt that comprises cationic lithium.

53. The method of claim 35 wherein the pericyte stressor comprises one or more agents selected from hydrogen peroxide, nitric oxide, tert-butylhydroperoxide, heavily-oxidized glycated LDL, and a pro-apoptotic agent.

54. The method of claim 35 wherein the level of pericyte loss comprises a level of one or more of pericyte cell death, pericyte apoptosis, pericyte necrosis, and pericyte autophagy.

55. The method of claim 35 wherein the level of pericyte dysfunction comprises a level of one or more of reactive oxygen species (ROS) production, reactive nitrogen species (RNS) production, matrix metalloproteinase 2 (MMP2) production, matrix metalloproteinase 9 (MMP9) production, angiopoietin 1 production, fibronectin 1 production, platelet derived growth factor receptor beta (PDGFRβ) expression, connexin 43 expression, NG2 expression, and IL-17R(A/C) expression.

56. The method of claim 35 wherein the candidate PPA is a cerebrospinal fluid (CSF) component that is produced by choroid plexus (CP) cells.

57. The method of claim 1 or claim 35 wherein prior to being cultured, the human CNS pericyte is obtained from a human having one of Parkinson's disease, Alzheimer's disease, Huntington's disease, and a retinal degenerative disease.

58. The method of claim 57 wherein the retinal degenerative disease is selected from macular degeneration, diabetic retinopathy, and retinitis pigmentosa.

59. A screening method for identifying a pericyte protective agent (PPA) that protects a peripheral nervous system (PNS) pericyte from a pericyte stressor, wherein said pericyte stressor is capable of inducing at least one of PNS pericyte loss and PNS pericyte dysfunction, the method comprising:

(a) contacting, simultaneously or sequentially and in any order,

(i) a cultured human PNS pericyte,

(ii) the pericyte stressor that is capable of inducing at least one of PNS pericyte loss and PNS pericyte dysfunction,

(iii) a conditioned culture medium that has been conditioned by mammalian choroid plexus (CP) cells and that contains a pericyte protective agent (PPA) produced by said CP cells, or an isolated fraction of said conditioned culture medium that contains the PPA, which PPA is capable of decreasing in the cultured human PNS pericyte a level of at least one of PNS pericyte loss and PNS pericyte dysfunction, relative to the level that is detected when the PPA is absent, and

(iv) one or a plurality of antibodies that have been generated against a portion of the culture medium that has been conditioned by mammalian choroid plexus (CP) cells and which contains the pericyte protective agent (PPA) produced by said CP cells,

under conditions and for a time sufficient to induce detectable PNS pericyte loss or detectable PNS pericyte dysfunction when the PPA is absent, thereby to obtain a human PNS pericyte test culture;

(b) detecting, in the human PNS pericyte test culture of (a), a level of at least one of PNS pericyte loss and PNS pericyte dysfunction that is decreased to a lesser degree relative to the level that is detected when

the one or plurality of antibodies is absent, thereby indicating that the one or plurality of antibodies is capable of neutralizing the PPA; and

(c) isolating the PPA by binding to at least one of the antibodies that is capable of neutralizing the PPA.

60. The method according to any preceding claim wherein the PPA comprises an H_2O_2-sensitive, thermostable, hexane-insoluble/ diethyl ether-insoluble/ ethyl acetate-insoluble and water-soluble molecule having a molecular weight of less than 3 kDa that is obtained in a culture medium that has been conditioned by cultured mammalian choroid plexus (CP) cells,

wherein the PPA is capable, upon being contacted with CNS pericytes, of decreasing a level of CNS pericyte loss or CNS pericyte dysfunction, relative to the level of CNS pericyte loss or CNS pericyte dysfunction when the PPA is absent.

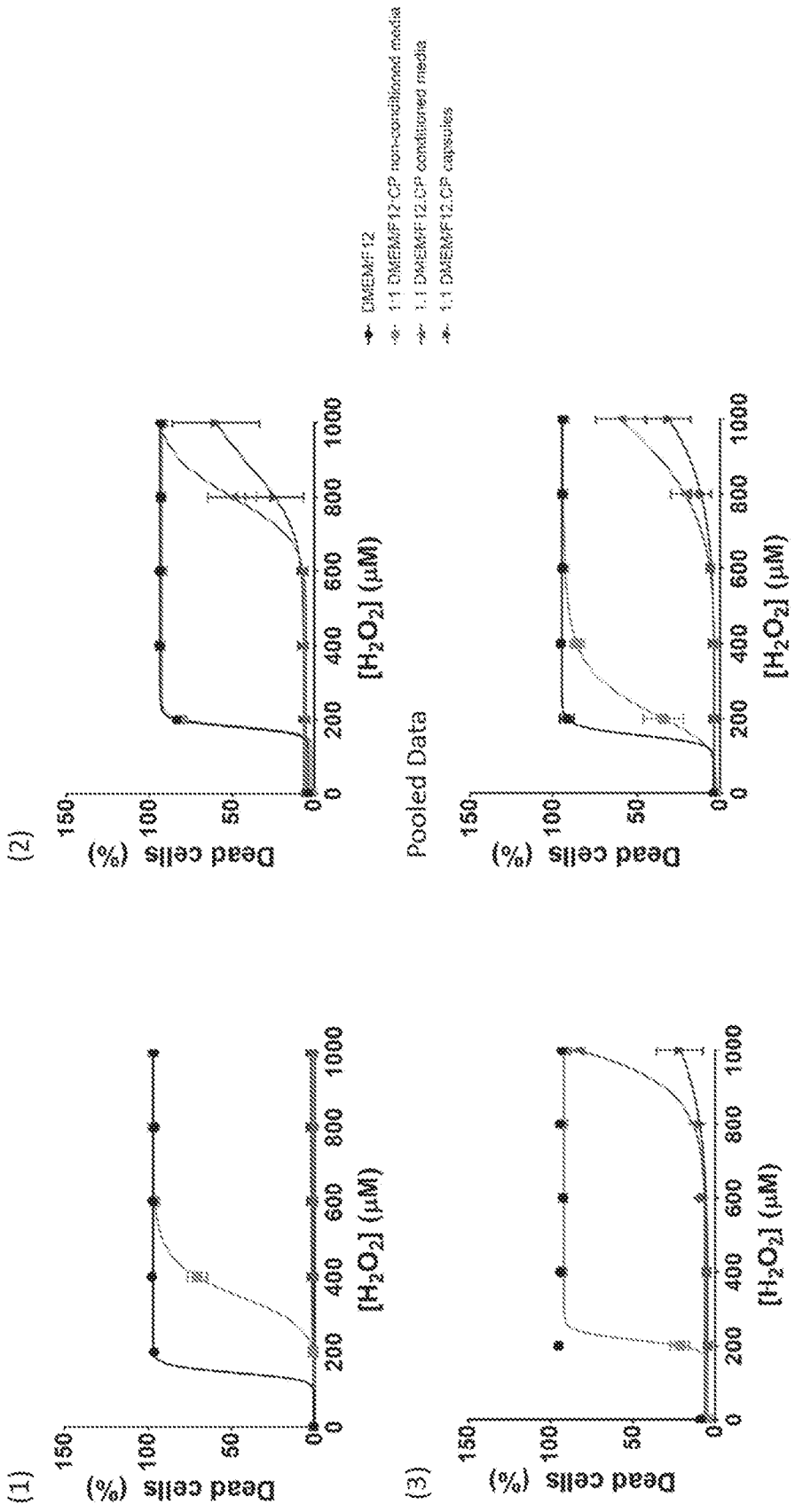

DMEM/F12
1:1 DMEM/F12:CP non-conditioned media
1:1 DMEM/F12:CP conditioned media
1:1 DMEM/F12:CP capsules
(1)
(2)
(3)
Pooled Data
Dead cells (%)
[H2O2] (µM)
0
50
100
150
200
400
600
800
1000

Fig. 1a

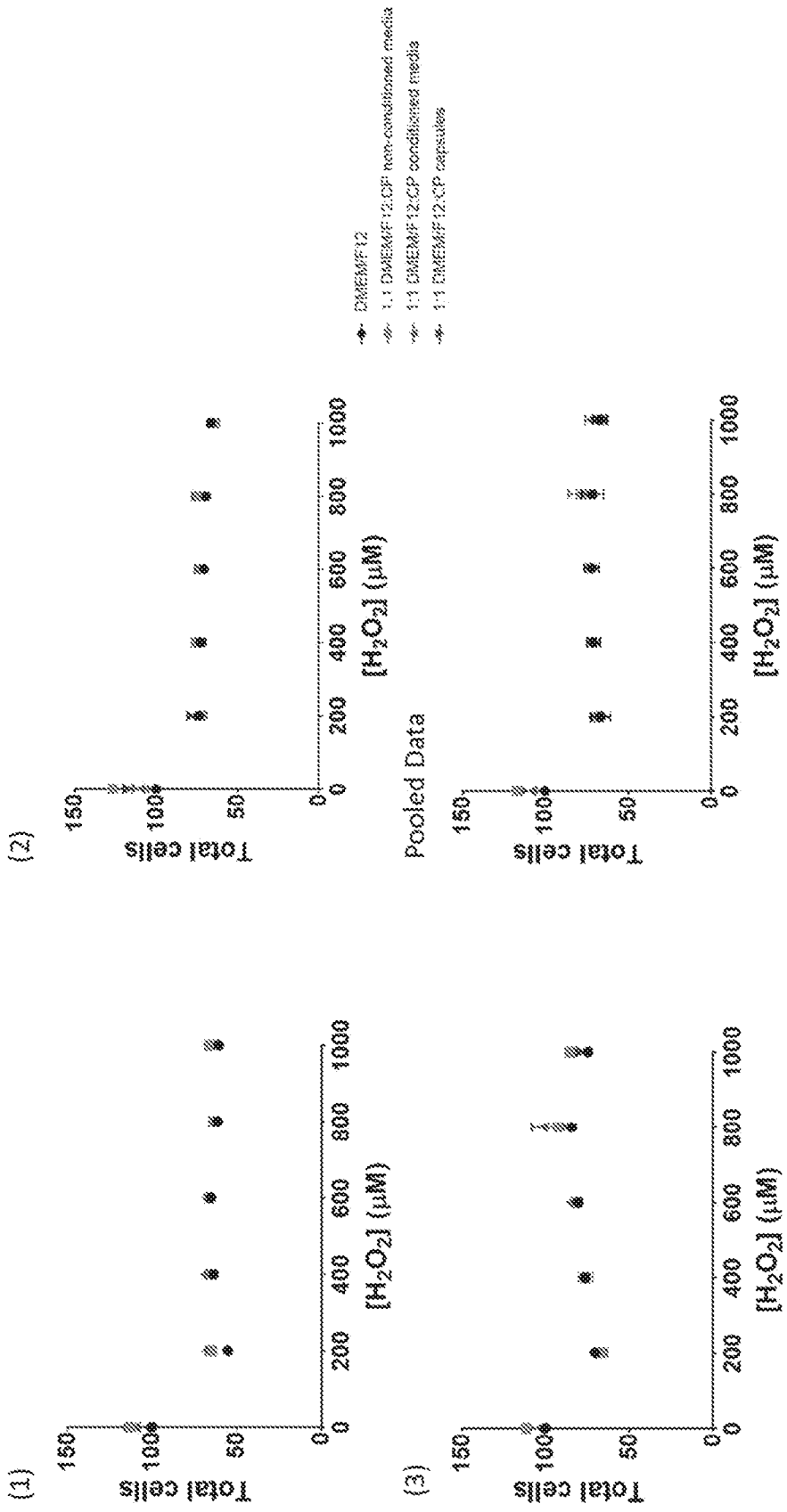

DMEM/F12
1:1 DMEM/F12:CP non-conditioned media
1:1 DMEM/F12:CP conditioned media
1:1 DMEM/F12:CP capsules
(1)
(2)
(3)
Pooled Data
Total cells
$[H_2O_2]$ (μM)
0
50
100
150
200
400
600
800
1000

Fig. 1b

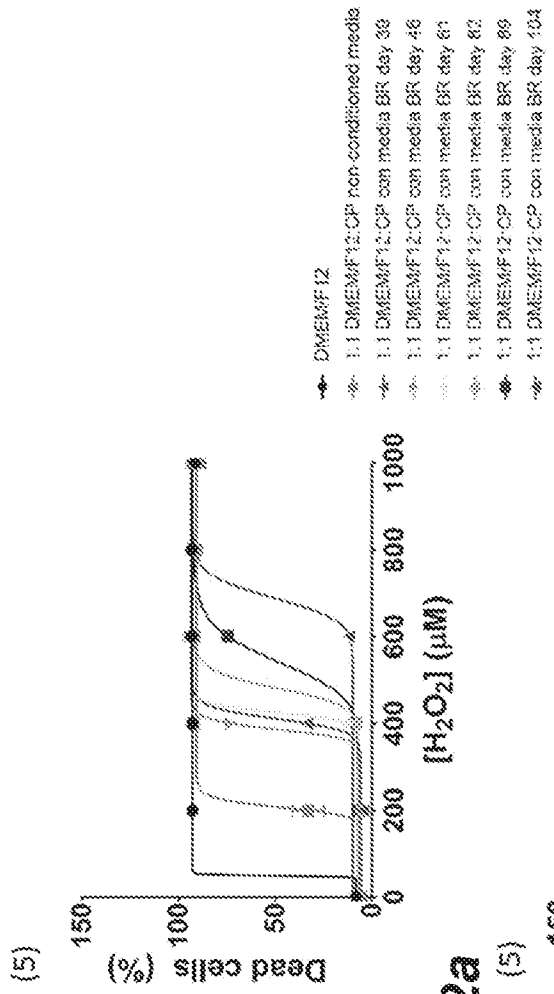

DMEM/F12
1:1 DMEM/F12:CP non conditioned media
1:1 DMEM/F12:CP con media BR day 39
1:1 DMEM/F12:CP con media BR day 46
1:1 DMEM/F12:CP con media BR day 61
1:1 DMEM/F12:CP con media BR day 82
1:1 DMEM/F12:CP con media BR day 89
1:1 DMEM/F12:CP con media BR day 104
(5)
Dead cells (%)
150
100
50
0
0
200
400
600
800
1000
[H2O2] (μM)

Fig. 2a

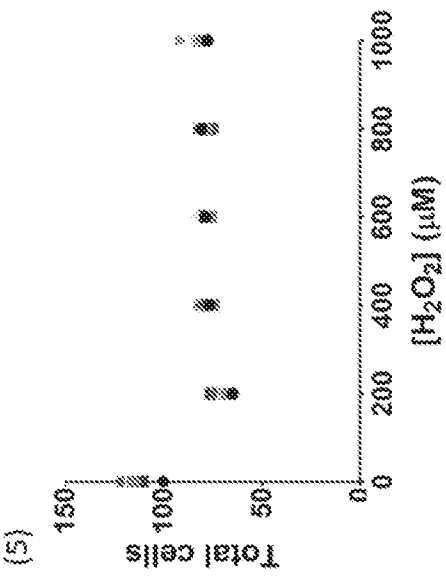
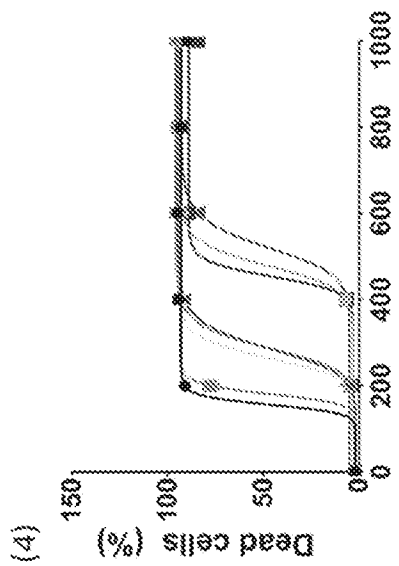
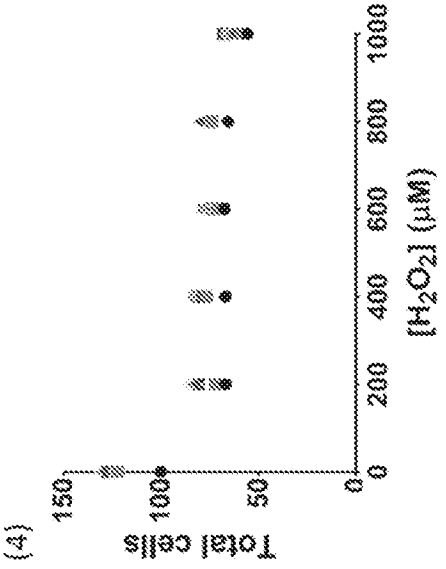

(5)
Total cells
150
100
50
0
0
200
400
600
800
1000
[H2O2] (μM)

Fig. 2b


(4)
Dead cells (%)
150
100
50
0
0
200
400
600
800
1000
[H2O2] (μM)


(4)
Total cells
150
100
50
0
0
200
400
600
800
1000
[H2O2] (μM)

CP Products/CSF Components

GENE/PROTEIN NAME	GENBANK® ACCESSION NUMBER; RELATED GENE ACCESSION NUMBER	SEQ ID NO:
Insulin / Insulin-like growth factors (IGFs)		
IGF-I	AY632379.1; NM_214256.1	
IGF-II	CK463136; BP152514; BP152514; BP142537; AF466293.1; NM_213883.1; BP442282	
Insulin-like growth factor binding protein 1 (IGFBP-1)	BX915622; BX916728	
Insulin-like growth factor binding protein 2 (IGFBP-2)	NM_214003.1	
Insulin-like growth factor-binding protein 3 (IGFBP-3)	AJ657291; AF085482.1	
insulin-like growth factor binding protein-5 (IGFBP-5)	NM_214099.1	
insulin-like growth factor binding protein 6 (IGFBP-6)	BX921939; CN163405	
Leydig insulin-like hormone	NM_213970.1	
insulin-degrading enzyme	CK451212; NP_004960.1	
Insulin-induced gene	BP454285	
Insulin (Preproinsulin)	AF064555.1	
Fibroblast growth factors (FGFs)		
Fibroblast growth factor 1 (FGF -1); acidic fibroblast growth factor	BF442355;	
Fibroblast growth factor 2 (FGF -2); basic FGF (bFGF)	AJ577089.1;	
Fibroblast growth factor 9 (FGF -9)	NM_213801.1	
Fibroblast growth factor 12 (FGF -12)	CO943884; NP_004104.3	
Fibroblast growth factor 18 (FGF -18)	CK457517; NP_032031.1	
Fibroblast growth factor 2-interacting factor 2; apoptosis inhibitor 5; API5-like 1	BI403651; XP_342471.1	
Transforming growth factor beta (TGF-β) superfamily		
Transforming growth factor beta 1 (TGF-β1)	CF180351;CF368013; NP_006013.1	
Transforming growth factor beta 2 (TGF-β2)	BQ604617; BP143282	

FIG. 3A

GENE/PROTEIN NAME	GENBANK® ACCESSION NUMBER; RELATED GENE ACCESSION NUMBER	SEQ ID NO:
Transforming growth factor beta 3 (TGF-β3)	BQ604617	
Transforming growth factor alpha	BP158556	
Transforming growth factor, beta-induced, 68kDa	CB478477; NP_000349.1	
Latent transforming growth factor beta binding protein 1	CO950891;NP_000618.1	
Latent transforming growth factor beta binding protein 4	BI182740; NP_003564.1	
Bone morphogenetic protein 1	BF079341; BI346244	
Bone morphogenetic protein 2	CA779719; AY669080.1	
Bone morphogenetic protein 4	CN159298; NP_001193.1	
Bone morphogenetic protein 7	CK452058; NP_001710.1	
Bone morphogenetic protein 11 (GDF11/BMP11)	AY669081.1; AF339155.1	
Bone morphogenetic protein 15	NM_001005155.1	
Growth differentiation factor 1	CK466140; NP_001483.2	
Growth differentiation factor 7	BQ604531; NP_878248.1	
Growth differentiation factor 8	NM_214435.2	
Growth differentiation factor 9	NM_001001909.1	
Growth differentiation factor 11 (GDF11)/ Bone morphogenetic protein 11 (BMP11)	AY669081.1; AF339155.1	
Vascular endothelial growth factor (VEGF)		
Vascular endothelial growth factor (VEGF)	CF789391; AY072734.1	
Vascular endothelial growth factor 2 (VEGF 2)	BI360137	
Vascular endothelial growth factor B (VEGF B)	CK453779; NP_003368.1	
Vascular endothelial growth factor C (VEGF-C)	BI404162; NP_446105.1	
Other factors		
Endozepine (DBI)	NM_214119.1	
Epidermal growth factor (EGF)	NM_214020.1	
Heparin-binding epidermal growth factor-like growth factor	NM_214299.1	
Growth hormone (GH)	NM_213869.1	
Nerve growth factor, beta (NGF-beta)	BX675501; NP_038637.1	

FIG. 3B

GENE/PROTEIN NAME	GENBANK® ACCESSION NUMBER; RELATED GENE ACCESSION NUMBER	SEQ ID NO:
Pigment epithelium derived factor (PEDF), or serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin), member 1 (Serpin F1)	BI181553; NP_002606.3	
Brain-derived neurotrophic factor (BDNF)	NM_214259.1	
Neurotrophin 3 (NT-3)	CF368973; NP_002518.1	
Neurotrophin 4 (NT-4)	T28141.1	
Metallothionein 3 (growth inhibitory factor (neurotrophic))	CF176439; NP_005945.1	
Glial cell-line derived neurotrophic factor (GDNF)	AB282866.1; GE904538.1; GE873770.1	
Connective tissue growth factor (CTGF)	BI181686; U83916.1	
Platelet derived growth factor, alpha	BX914490; NP_032834.1	
Keratinocyte growth factor	AF217463.1	
Heparin-binding epidermal growth factor-like growth factor (DTR)	NM_214299.1	
Axotrophin	CF175198; NP_073737.1	
Neurite growth-promoting factor-2/ Midkine	CN166538; NP_002382.1	
Growth factor, augmenter of liver regeneration (ERV1 homolog)	CN154812; NP_005253.2	
Hepatoma-derived growth factor (high-mobility group protein 1-like)	CN160438; NP_004485.1	
Hepatoma-derived growth factor-related protein 2	BX924740; NP_116020.1	
Macrophage stimulating 1 (Hepatocyte growth factor-like)	CK459058; NP_066278.2	
Teratocarcinoma-derived growth factor 1	CN163564;NP_003203.1	
Apoptosis inhibitor 5; fibroblast growth factor 2-interacting factor 2; API5-like 1	BI403651;XP_342471.1	
Growth arrest and DNA-damage-inducible, beta	BF710383; CF175279; NP_056490.1	
Growth arrest and DNA-damage-inducible protein GADD45, gamma	BX675976; XP_237999.2	
Growth arrest-specific 2	CK464946; NP_005247.1	
Growth arrest homeobox transcription factor GAX (GAX)	NM_214121.1	
Inhibitor of growth family, member 1-like	AW786251;NP_075992.2	
Inhibitor of growth family, member 3	CN163027; NP_061944.2	

FIG. 3C

GENE/PROTEIN NAME	GENBANK® ACCESSION NUMBER; RELATED GENE ACCESSION NUMBER	SEQ ID NO:
Pregnancy-induced growth inhibitor	CN159080; NP_892025.1	
Metallothionein 3 (growth inhibitory factor (neurotrophic)	CF176439;NP_005945.1	
RAS-like, estrogen-regulated, growth inhibitor	BX666549; NP_116307.1	
Cytokine induced apoptosis inhibitor 1	CK451092;NP_598902.1	
Antioxidants		
Ceruloplasmin	BF713714	
Superoxide dismutase-1 (SOD-1)	CO992469	
Superoxide dismutase-2 (SOD-2), Mn-type	BI340469; NM_214127.1	
Superoxide dismutase copper chaperone (CCS)	NM_001001866.1	
DJ-1/PARK7	CK456067; NP_009193.2	
Catalase	NM_214301.1	
Selenoprotein I, 1	CN167111; XP_371484.1	
Selenoprotein M	CN162429	
Selenoprotein N	CN167189; XP_342943.1	
Selenoprotein P	CF175878;(NP_005401.2)	
Selenoprotein S	CF787953; NP_060915.2	
Selenoprotein T	BI399114; NP_057359.1	
Selenoprotein W	NM_213977.1	
Selenoprotein X, 1	CK462523; NP_057416.1	
Selenoprotein, 15 kDa	CB477630; NP_004252.1 15	
Glutathione S-transferase	CO945521	
Glutathione S-transferase (GSTA2)	NM_214389.1	
Glutathione S-transferase (LOC396850)	NM_213850.1	
Glutathione S-transferase (MGST1)	NM_214300.1	
Glutathione S-transferase, alpha 4	CK455717; NP_034487.1	
Glutathione S-transferase A4	CN159152; NP_001503.1	
Glutathione S-transferase kappa 1	CN167195; NP_057001.1	
Glutathione S-transferase M1	CF180873; NP_000552.2	
Glutathione S-transferase, mu 5	CN028399; NP_034490.1	
Glutathione S-transferase omega (GSTO1)	NM_214050.1	
Glutathione S-transferase omega 2	CK467367; NP_899062.1	
Glutathione S-transferase, pi 2	CN154388; NP_620430.1	
Glutathione S-transferase theta 1	BF080536; CA781059; NP_000844.1	

FIG. 3D

GENE/PROTEIN NAME	GENBANK® ACCESSION NUMBER; RELATED GENE ACCESSION NUMBER	SEQ ID NO:
Glutathione transferase zeta 1 (maleylacetoacetate isomerase)	CF789130; AW415841; NP_034493.1	
Epididymis-specific glutathione peroxidase 23kDa subunit (GPX5)	NM_213886.1	
Microsomal glutathione S-transferase 2	CF789192; NP_002404.1	
Microsomal glutathione S-transferase 3	CK466828	
Glutathione peroxidase 2	CF365816; XP_238459.2	
Glutathione peroxidase 3	BX671405	
Glutathione peroxidase 4	BI183078	
Glutathione peroxidase 7	BI183697; CN159630; NP_056511.2	
Glutathione peroxidase 6 (olfactory)	CK456244; NP_874360.1	
Cytosolic glutathione peroxidase (GPX1)	NM_214201.1	
Thioredoxin (TXN)	NM_214313.1	
Glutathione reductase	CB471915	
Glutathione reductase 1	CK461867; NP_034474.3	
Hydroxyacyl glutathione hydrolase	BF078108; CF179717; NP_077246.1	
Chemotactic factors		
Alveolar macrophage-derived chemotacticfactor-I	NM_213867.1	
Alveolar macrophage-derived chemotactic factor-II (AMCF-II)	BQ597577; NM_213876.1	
Eosinophil chemotactic cytokine	CF368668	
Stromal cell-derived factor 2	CO950580; NP_008854.2	
Stromal cell-derived factor 2-like 1	CK456849; NP_071327.1	
Chemokine ligand 2	BF078671; NM_001001861.1	
CCL 8 Chemokine, (C-C motif) ligand 8	BI399438; NP_005614.2	
CCL16 Chemokine, (C-C motif) ligand 16	BX922646; NP_004581.1	
CCL19 chemokine (CCL19)	BX672579	
CCL21 chemokine (CCL21)	AY312067.1	
CCL25 chemokine (CCL25)	BP164490	
CXCL2 chemokine (C-X-C motif) ligand 2	AJ747030; CB472456; NP_002080.1	
CXCL4 chemokine, (C-X-C motif) ligand 4	CK457066; (NP_064316.1)	
CXCL9 chemokine (C-X-C motif) ligand 9	BX914993; NP_002407.1	
CXCL12 chemokine (CXCL12)	AY312066.1	
CXCL13 chemokine, (C-X-C motif) ligand 13 (B-cell chemoattractant)	BI336231; CF787657; NP_006410.1	

FIG. 3E

GENE/PROTEIN NAME	GENBANK® ACCESSION NUMBER; RELATED GENE ACCESSION NUMBER	SEQ ID NO:
CXCL14 chemokine (CXCL14)	BF192019	
Chemokine-like factor (CKLF) super family 3	BI402082; CB483090; CN069866; NP_653202.1	
Chemokine-like factor (CKLF) super family 6	AJ651390; (NP_060271.1)	
Chemokine-like factor (CKLF) super family 7	CN165006; NP_612419.1	
Tachykinin, precursor 1 (substance K, substance P, neurokinin 1, neurokinin 2, neuromedin L, neurokinin alpha, neuropeptide K, neuropeptide gamma)	BF441434; NP_054703.1	
Neurite growth-promoting factor-2/Midkine	CN166538; NP_002382.1	
Neuropeptide Y	AF264083.1;	
Neuronal and endocrine protein (7B2)	M23654.1	
Neuromedin B	CN156167; NP_066563.1	
Chaperone proteins		
Transthyretin	NM_214212.1	
Apolipoprotein A-I (APOA1)	NM_214398.1	
Apolipoprotein A-I binding protein	CK457272; NP_658985.1	
Apolipoprotein A-IV (APOA4)	NM_214388.1	
Apolipoprotein A-II	BF702658; NP_001634.1	
Apolipoprotein B (apoB)	AJ399510.1; M62614.1	
Apolipoprotein B (including Ag(x) antigen)	CK455263;CF176070; NP_000375.1	
Apolipoprotein C-II	CK455497; NP_000474.2	
Apolipoprotein C-III (APOC3)	NM_001002801.1	
Apolipoprotein D	BX667312; NP_001638.1	
Apolipoprotein E (APOE)	NM_214308.1	
Apolipoprotein H (beta-2-glycoprotein I)	BX672492; NP_000033.1	
Apolipoprotein M	BI341006; NP_061974.2	
Apolipoprotein N (ApoN)	AY583018.1	
Apolipoprotein R (C4BPA)	NM_213942.1	
Apolipoprotein J/clusterin	DR003806; DN990822.1; T03485.1; T18966.1; T15710.1; M78173.1; M78172.1; M78214.1	
Lipocalin 6	BX915534; (NP_945184.1)	
Lipocalin 7	BI399153; NP_071447.1	
Salivary lipocalin (SAL1)	NM_213814.1	

FIG. 3F

GENE/PROTEIN NAME	GENBANK® ACCESSION NUMBER; RELATED GENE ACCESSION NUMBER	SEQ ID NO:
Tear lipocalin (LCN1)	NM_213856.1	
Heat shock proteins		
Heat shock protein 1, alpha	BI399688; NP_786937.1	
Heat shock 10kD protein (HSPE1)	NM_214307.1	
Heat-responsive protein 12	CK451954; NP_005827.1	
Heat shock 27kDa protein 1	CK461732; NP_001531.1	
Heat shock 27kDa protein 2	BM083187; BX670646; NP_001532.1	
Heat shock 27kDa protein 3	BX922244; NP_006299.1	
Heat shock 27kDa protein family, member 7 (cardiovascular)	BM190065; NP_055239.1	
Heat shock 40kD protein 1	BG894473;	
Heat shock protein 47	CN160329;	
Heat shock 60kDa protein 1 (chaperonin)	CB469603; NP_002147.2	
Heat shock protein 67B2	BX920632; XP_376522.1	
Heat shock 70kDa protein 1B	CN159038; NP_005337.1	
Heat shock 70kDa protein 2	BF704466; NP_068814.2	
Heat shock protein 70.2 (HSP70.2)	NM_213766.1	
Heat shock 70kDa protein 4	CA780945; BF709866; BQ603676; NP_002145.3	
Heat shock 70kDa protein 5	AJ659816	
Heat shock 70kDa protein 8	CK462212; NP_006588.1	
Heat shock 70kDa protein 9B (mortalin-2)	AJ667285; BQ604492; NP_004125.3	
Heat shock cognate 71 kDa protein	BF703190; XP_214603.1	
Heat shock 90kDa protein (HSP90)	CF180819; BQ604703; NM_213973.1	
Heat shock 90kD protein 1, beta	CN166333	
Heat shock 105kDa110kDa protein 1	CO993113; NP_006635.2	
Heat shock protein, alpha-crystallin-related, B6	CF180865; NP_653218.1	
Calcium regulated heat stable protein 1	BX915555; NP_690003.1	
Heat shock factor binding protein 1	BX920160; CF365327; NP_001528.1	
Heat shock factor binding protein 1	BI181454; NP_001528.1	
Heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) binding protein 1	CK456939; NP_821174.1	

FIG. 3G

GENE/PROTEIN NAME	GENBANK® ACCESSION NUMBER; RELATED GENE ACCESSION NUMBER	SEQ ID NO:
Other chaperone proteins		
Park2 co-regulated	BF711363; (XP_128418.1)	
p53-associated parkin-like cytoplasmic protein	CF362946; NP_055904.1	
Parkinson disease (autosomal recessive, early onset) 7 (PARK7/DJ-1)	CK456067; NP_009193.2	
Adipocyte fatty acid-binding protein	AU059657	
Fatty acid-binding protein, epidermal (E-FABP) (Psoriasis-associated fatty acid-binding protein homolog) (PA-FABP)	CB468944; XP_370729.1	
Liver fatty acid binding protein	BX924285	
Fatty acid binding protein 2, intestinal	CB285696; NP_000125.1	
Fatty acid binding protein 7, brain	CK463743; NP_001437.1	
Delta 5 fatty acid desaturase	AW346753; NP_835229.1	
Delta-6 fatty acid desaturase	CF791131	
Prostaglandin-endoperoxide synthase 1 (prostaglandin GH synthase and cyclooxygenase)	BP447300; CK452098; NP_542158.1	
prostaglandin D synthase (PTGDS)	NM_214228.1	
prostaglandin E (synthase)	CK465313; NP_004869.1	
prostaglandin GH synthase-2 (PGHS-2)	NM_214321.1	
beta-amyloid binding protein precursor	CN157258; NP_114416.1	
Amyloid prcursor protein	NM_214372.1	
Adrenomedulin	NM_214107.1	
Amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe65)	AW431342; NP_001155.	
Amyloid beta (A4) precursor-like protein 2	BX672465; BF703315; CA780698; NP_001633.1	
Amyloid beta precursor protein binding protein 1, 59kDa	CK452261; NP_003896.1	
Amyloid beta precursor protein (cytoplasmic tail) binding protein 2	AJ655868; NP_006371.2	
Serum amyloid A4, constitutive	CN070401; NP_006503.1	
Serum amyloid P component (SAP)	AB005546.1;	
Serum amyloid A 3	CB475095; NP_035445.1	
Islet amyloid polypeptide	BF712755; (NP_036718.1)	
Synuclein, alpha interacting protein (synphilin)	BX923294; NP_005451.1	

FIG. 3H

GENE/PROTEIN NAME	GENBANK® ACCESSION NUMBER; RELATED GENE ACCESSION NUMBER	SEQ ID NO:
Prion protein interacting protein	CN160527; XP_290941.2; NP_076971	
Proteinase inhibitors		
Serine (or cysteine) proteinase inhibitor, clade A (alpha-1antiproteinase, antitrypsin), member 1 (PI)	NM_214395.1;	
Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6	AJ683219; NP_004559.4	
Serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1, (angioedema, hereditary)	CN153484; NP_000053.1	
Serine (or cysteine) proteinase inhibitor, clade I (neuroserpin), member 1	BI404568 ;NP_005016.1	
Cystatin C (amyloid angiopathy and cerebral hemorrhage)	CF180804;(NP_000090.1)	
Cystatin B (stefin B)	CN164516; NP_000091.1	
Cystatin EM	CK463154; NP_001314.1	
Cystatin 11	BX674305; XP_283777.1	
Cystatin 9-like	CF365609; NP_542177.1	
Metalloproteinase tissue inhibitor 1	NM_213857.1	
Tissue inhibitor of metalloproteinase-3	AF156031.1;BF711174; NP_037018.1	
Matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	BX922846; NP_004985.1	
Cytochrome P450 enzymes (Drug and steroid metabolism, steroid biosynthesis enzymes)		
Cytochrome P450 1A1 (CYP1A1)	NM_214412.1	
Coumarin 7-hydroxylase (CYP2A)	AY280866.1	
Cytochrome P450 2B22 (CYP2B22)	NM_214413.1	
Cytochrome P450 2C32 (CYP2C32)	U35733.1	
Cytochrome P450 2C33 (CYP2C33)	NM_214414.1	
Cytochrome P450 2C36 (CYP2C36)	BX668048	
Cytochrome P450 C42 (CYP2C42)	Z93098.1	
Cytochrome P450 2C49 (CYP2C49)	NM_214420.1	
Vitamin D3 25-Hydroxylase (CYP2D25)	NM_214394.1	
Vitamin D3 25-Hydroxylase (CYP2D25)	CN159866	

FIG. 3I

GENE/PROTEIN NAME	GENBANK® ACCESSION NUMBER; RELATED GENE ACCESSION NUMBER	SEQ ID NO:
Cytochrome P450, family 2, subfamily E, polypeptide 1 (CYP2E1)	NM_214421.1	
CYP3A22 mRNA for cytochrome P450 (CYP3A22)	AB006010.1; BI182277	
Cytochrome P450 3A29	NM_214423.1	
Cytochrome P450 3A39 (CYP3A39)	NM_214422.1	
Cytochrome P450 3A46	AB052266.1; F22946	
Cytochrome P450 4A24 (CYP4A24)	NM_214424.1	
Cytochrome P450 4A21 (CYP4A21)	NM_214425.1	
Cholesterol 7alpha-hydroxylase (CYP7)	NM_001005352.1	
Cytochrome P-450 8B1 (CYP8B1)	NM_214426.1	
Cytochrome P450 11A1 (CYP11A1)	NM_214427.1	
Cytochrome P450 17A1 (CYP17A1)	NM_214428.1	
CYP19, aromatase P450	S80147.1; S80148.1; U37311.1	
Cytochrome P450 19A1/ ARORA1-5 cytochrome P450 aromatase	U37310.1	
Cytochrome P450 19A3 (CYP19A3)	NM_214431.1	
25-hydroxyvitamin D3-24-hydroxylase (CYP24A1)	NM_214075.1	
25-hydroxyvitamin D3 1alpha-hydroxylase (CYP27B1)	NM_213995.1	
Cytochrome P450 51 (CYP51)	NM_214432.1	

FIG. 3J

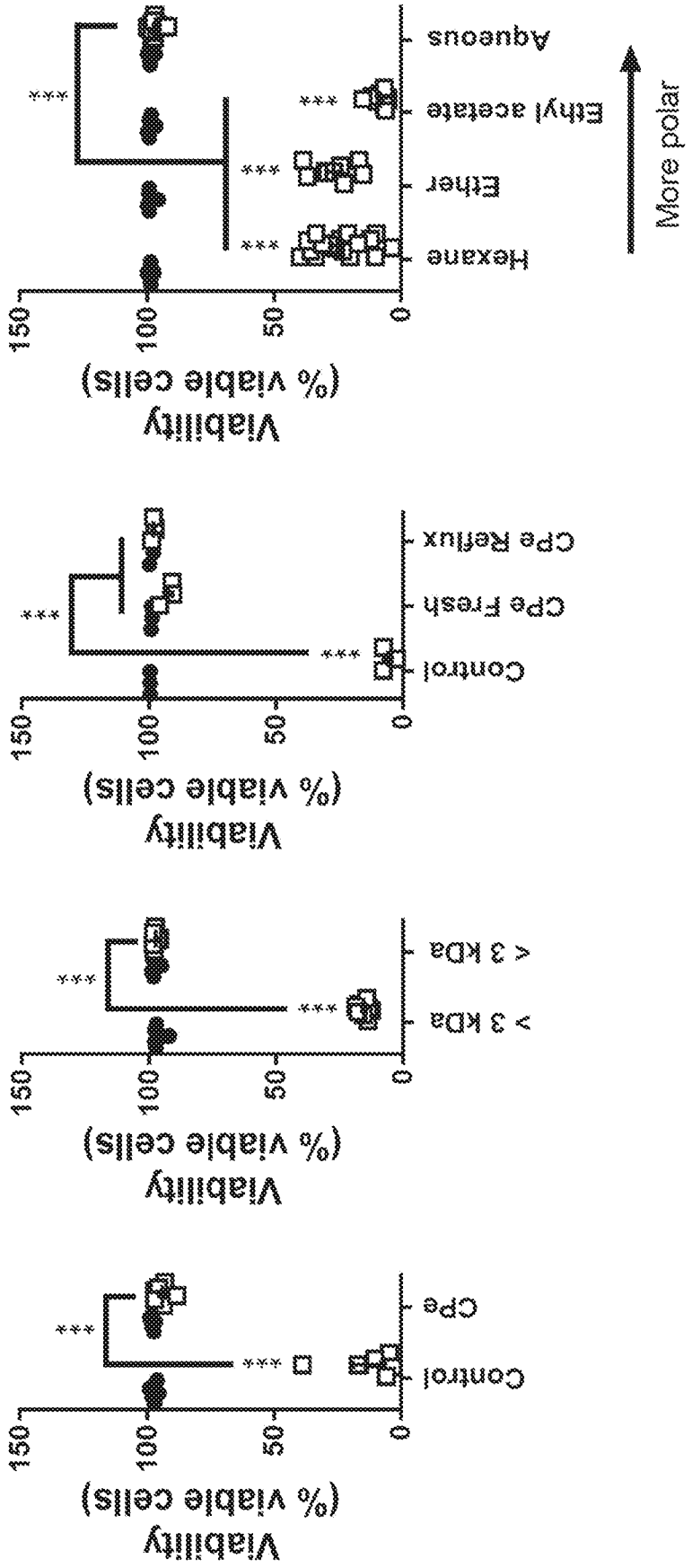

Viability
(% viable cells)
150
100
50
0
Control
CPe
***
***
Viability
(% viable cells)
> 3 kDa
< 3 kDa
***
***
Viability
(% viable cells)
Control
CPe Fresh
CPe Reflux
***
***
Viability
(% viable cells)
Hexane
Ether
Ethyl acetate
Aqueous
More polar
***
***
***

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/58797

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 35/30, C12Q 1/68 (2019.01)
CPC - A61K 35/30, C12N 5/0012, C12N 5/0622, A61K 2035/128, C12Q 1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2011/0206612 A1 (MARTIN et al.,) 25 August 2011 (25.08.2011) Abstract; para [0003]; para [0021]; para [0025]; para [0030]; para [0068-0069]; para [0074-0075]; para [0165-0166]; para [0172]; para [0231]	1-23, 35-59
Y	WO 2015/107196 A1 (INSTITUT CURIE et al.) 23 July 2015 (23.07.2015) Abstract; p10, ln 11-20; p13, ln 34-p14; p15, ln 8-9; p17, ln 13-16	1-23, 55, 57/1, 58/1
Y	US 2016/0361365 A1 (LIVING CELL TECHNOLOGIES NEW ZEALAND LIMITED) 15 December 2016 (15.12.2016) Abstract; Claim 7; Claim 10; para [0005]; para [0012]; para [0015];para [0031]; para [0064]; para [0083]; para [0089]; para [0093]; para [0141]	3-19, 23, 35-56, 57/35, 58/35, 59
Y	US 8,119,609 B2 (LECOMTE et al.,) 21 February 2012 (21.02.2012) col 5, ln 10-15	20, 21, 53, 54

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 February 2019	25 FEB 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/58797

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 60
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
----Please see continuation in first extra sheet ----------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-23, 35-59

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/58797

Continuation of Box No. III. Observations where unity of invention is lacking.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: claims 1-23, 35-59, drawn to a screening method for identifying a pericyte protective agent (PPA) that protects a central nervous system (CNS) pericyte from a pericyte stressor.

Group II: claims 24-34, drawn to a method for treating a central nervous system (CNS) condition associated with CNS pericyte loss or CNS pericyte dysfunction in a subject.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I includes the special technical feature of a method comprising contacting pericyte culture with a stressor and a pericyte protective agent, not required by Group II.

Group II includes the special technical feature of a method comprising administering to a subject a pericyte protective agent, not required by Group I.

Common Technical Features

The inventions of Groups I and II share the technical feature of a method comprising under conditions that permit the PPA to contact CNS pericytes in the subject to decrease a level of CNS pericyte loss or CNS pericyte dysfunction in the subject relative to the level of CNS pericyte loss or CNS pericyte dysfunction in the subject when the PPA is absent.

However, these shared technical features do not represent a contribution over prior art in view of US 2011/0206612 A1 to Martin et al., (hereinafter 'Martin') and WO 2015/107196 A1 to Institut Curie et al., (hereinafter 'Institute Curie').

Martin teaches (instant claim 1) a screening method for identifying a pericyte protective agent (PPA) that protects a central nervous system (CNS) pericyte from a pericyte stressor, wherein said pericyte stressor is capable of inducing at least one of CNS pericyte loss and CNS pericyte dysfunction (Abstract - 'The present invention provides methods for determining or identifying compounds that modulate the function of an isolated retinal pericyte or blood vessel, wherein a change in the contractile state of said pericyte or blood vessel is determined in the presence of a test compound, said change indicating that the test compound modulates the function of pericytes and/or blood vessels.'; para [0003] - 'Further the present invention relates to novel screens for agents that modulate pericyte function, such as, for example, the contraction of pericytes, cell growth, differentiation, ion channel conductivity, neurotransmitter release, or gene transcription.'), the method comprising:
(a) contacting, simultaneously or sequentially and in any order, (i) a cultured CNS pericyte with (ii) the pericyte stressor that is capable of inducing CNS pericyte dysfunction, and (iii) a candidate pericyte protective agent (PPA), under conditions and for a time sufficient to induce detectable CNS pericyte dysfunction when the PPA is absent, thereby to obtain a human CNS pericyte test culture; and
(b) detecting, in the CNS pericyte test culture of (a), a level of CNS pericyte dysfunction that is decreased relative to the level that is detected when the PPA is absent, and thereby identifying the candidate PPA as a pericyte protective agent (PPA) (para [0068] - 'The present invention provides methods for determining or identifying compounds that modulate pericyte function, wherein a change in the contractile state of a pericyte is determined in the presence of a test compound, said change indicating that the test compound modulates pericyte function.; para [0069] - 'In one embodiment, the method comprises incubating the pericyte in the presence of a test compound.'; para [0231] - 'The present inventors further identified protein kinase A (PKA) as being affected by the increase in cAMP following the binding of PACAP or VIP. For this purpose cells were grown on silicone substrate and contacted by a 10.sup.-8 M concentration of PACAP. The drug adenosine 3',5'-cyclic monophosphothioate, Rp-isomer (Rp-cAMPS, A7850 Sigma USA) is a specific inhibitor of PKA activity and was added sequentially into the PACAP 10.sup.-8 M solution in three separate concentrations....We discovered that Rp-cAMPS, in a dose-dependent manner and with an EC50 value of 26 uM, inhibited the relaxing effect of 10.sup.-8M PACAP. It was shown that the drug N-(2-[p-bromocinnamylamino]-ethyl)-5-isoquinolinesulfonamid-e (H-89, B 1427 Sigma USA) at a concentration of 0.3 uM, which is another specific inhibitor of PKA activity, inhibited the relaxing effect of 10.sup.-8 M PACAP. See FIG. 6.').
Martin does not expressly teach said agent is a pericyte protective agent, or pericyte is a human CNS pericyte.
Institut Curie teaches a pericyte protective agent for human pericytes (Abstract - 'The present invention relates to the use of thalidomide or analogs thereof for preventing neurologic disorders induced by brain irradiation.'; p10, ln 11-20, - 'The present invention relates to CNS or brain tumor....The patient is a human being. '; p13, ln 34-p14, ln 1 - 'Figure 2. Irradiation induces changes in pericytes phenotype, which is prevented by peri-irradiation treatment with thalidomide'; p15, ln 8-9, - 'Figure 5: Pericyte-mediated vessel constriction is impaired in irradiated mice, an effect reversed by pharmacological treatment with thalidomide'). Since Institut Curie teaches thalidomide protects pericyte dysfunction following radiation stress, it would have been obvious to one of ordinary skill in the art that Thalidomide is a pericyte protective agent (PPA) for human CNS pericytes, and the screening method of Martin could be used to screen human pericytes to identify PPA according to Institut Curie.

---continued on next sheet---

Continuation of Box No. III. Observations where unity of invention is lacking.

Continuation from prior sheet

The inventions of Groups I and II share the technical feature of a pericyte protective agent produced by a choroid plexus (CP) composition which is diclosed by US 2016/0361365 A1 to Living Cell Technologies New Zealand Limited (hereinafter 'LCT') teaches a pericyte protective agent produced by a choroid plexus (CP) composition (abstract, 'Compositions and methods are disclosed that relate to improved treatments for nervous system diseases and disorders using CNS-implanted semi-permeable biocompatible capsules containing encapsulated pathogen-free xenogeneic choroid plexus (CP) cells that are induced to produce altered (and in certain embodiments increased) levels of one or more cerebrospinal fluid (CSF) components.').

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Groups I and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Item 4 (continued) Claim 60 is a dependent claim and is not drafted in accordance with the second and third sentences of Rule 6.4(a).