

and impaired neural function. As a result, many human diseases are associated with a loss or reduction in myelination, such as multiple sclerosis or white vanishing matter disease.

[0005] The study of myelination in health and disease is limited by the availability of appropriate models. Rodents are commonly used as a surrogate to study aspects of human biology; however, myelination is much more extensive in human than in rodent brains. Additionally, limited availability of healthy brain samples from human patients precludes the possibility of extensively studying myelination in the human brain. In recent years, attempts have been made to generate oligodendrocytes progenitor cells (OPC) and oligodendrocytes from human embryonic and induced pluripotent stem cells (hESC, hiPSCs) for the study of myelination *in vitro*.

[0006] hESC and hiPSC-derived models of myelination are not only essential to the study the processes of oligodendrocyte development and myelination under normal and disease conditions, but can also be applied for pharmaceutical drug screening. These models can be used to both screen for compounds that correct specific abnormalities related to certain disease states and to test the toxicity of new therapeutic compounds and chemical substances prior to human exposure. Particularly, in the field of neurotoxicity, assays capable of assessing the impairment of neuronal or glial function are still lacking for human cells.

[0007] Therefore, the development of *in vitro* screening platforms that recapitulate highly functional human tissue, including myelination of neurons, is of utmost importance.

[0008] Publications. Methods to reprogram primate differentiated somatic cells to a pluripotent state include differentiated somatic cell nuclear transfer, differentiated somatic cell fusion with pluripotent stem cells and direct reprogramming to produce induced pluripotent stem cells (iPS cells) (Takahashi K, et al. (2007) Cell 131:861-872; Park IH, et al. (2008) Nature 451:141-146; Yu J, et al. (2007) Science 318:1917-1920; Kim D, et al. (2009) Cell Stem Cell 4:472-476; Soldner F, et al. (2009) Cell. 136:964-977; Huangfu D, et al. (2008) Nature Biotechnology 26:1269-1275; Li W, et al. (2009) Cell Stem Cell 4:16-19).

SUMMARY

[0009] Compositions and methods are provided for *in vitro* generation of human OPCs and myelinating oligodendrocytes, which may be cultured in a 3-dimensional system with central nervous system neurons. A feature of the disclosure is the ability to generate OPCs and oligodendrocytes from patient samples, allowing disease-relevant generation and screening of the cells for therapeutic drugs and treatment regimens. The methods utilize induced human

pluripotent stem cells (hiPSCs), which may be obtained from patient or carrier cell samples, e.g. adipocytes, keratinocytes, fibroblasts, and the like. The hiPSCs are induced to develop an ectoderm fate *in vitro* and subsequently differentiated into spheroids that contain oligodendrocytes- human oligodendro-spheroids (hOS), as well as neural progenitors, astrocytes and neurons. The cell populations can be isolated from the hOS, or the intact hOS can be used as a model for interacting cell populations. The hOS and cells derived therefrom may be used for transplantation, for experimental evaluation, as a source of lineage and cell specific products, and the like. In some examples the cell cultures are feeder-free and xeno-free.

[0009a] In a first aspect, the present disclosure refers to a method for producing human oligodendrocytes *in vitro*, the method comprising: inducing in a pluripotent stem cell suspension culture a neural fate to provide a spheroid of neural progenitor cells; differentiating the neural progenitor cells in a spheroid to differentiate into human oligodendro-spheroids (hOS) including oligodendrocytes; maintaining the hOS spheroid for an extended period of time in neural medium to derive cultures that include myelinating oligodendrocytes, neurons and astrocytes.

[0009b] In a second aspect, the present disclosure refers to a population of oligodendrocytes obtained by the method disclosed herein.

[0010] In some examples of the disclosure, populations of purified human OPC or oligodendrocytes are provided, including without limitation disease-relevant oligodendrocytes, where the cells are differentiated from induced human pluripotent stem cells (hiPSCs). In some examples a panel of such *in vitro* derived cells are provided, where the panel includes two or more genetically different cells. In some examples a panel of such cells are provided, where the cells can be subjected to a plurality of candidate agents, or a plurality of doses of a candidate agent. Candidate agents include small molecules, i.e. drugs, genetic constructs that increase or decrease expression of an RNA of interest, electrical changes, and the like. In some examples a panel refers to a system or method utilizing patient-specific cells from two or more distinct conditions, and may be three or more, four or more, five or more, six or more, seven or more genetically distinct conditions.

[0011] In some examples of the disclosure, methods are provided for determining the activity of a candidate agent on oligodendrocytes from the hOS, the method comprising contacting the candidate agent with one or a panel of purified neurons, astrocytes, OPs or oligodendrocyte populations differentiated from induced human pluripotent stem cells (hiPSCs). The cell populations optionally comprise at least one allele encoding a mutation associated or causing a myelinating or demyelinating disease or an oligodendrocyte development disorder; and determining the effect of the agent on morphologic, genetic or functional parameters, including without limitation gene expression profiling. In a third aspect, the present disclosure refers to a method determining the effect of a candidate agent on a human oligodendrocytes, the method comprising: contacting the candidate agent with one or a panel of astrocytes differentiated from induced human pluripotent stem cells (hiPSC) according to the method of the first aspect, wherein the oligodendrocytes optionally comprise at least one allele encoding a mutation associated with a neural disease; and determining the effect of the agent on morphologic, genetic or functional parameters. The screening methods may be combined with immune effector cells to determine activity of such immune cells of oligodendrocytes and myelination, or the interaction between oligodendrocytes, astrocytes in hOS and autologous immune cells from patients. Methods of analysis at the single cell level are of particular interest, e.g. myelination assays, single cell gene expression, effect of oligodendrocytes on neurons or astrocytes, and neuronal cell signal transmission, and the like. Candidate agents include immune effector cells, e.g. T cells, microglial cells, macrophages, NK cells, etc., and immune

effector proteins, e.g. IFN- γ , TGF- β , cytokines, interferons, etc. and the like, particularly such cells and proteins suspected of involvement in inflammatory demyelinating diseases.

[0012] The methods of the disclosure utilize the natural interactions between neural cells and progenitors in the differentiation of oligodendrocytes and formation of myelin sheaths. In some examples, the differentiation from hiPSCs to oligodendrocytes is performed in substantially serum-free medium.

[0013] After differentiation in hOS, individual cell types of interest, including and without limitation to oligodendrocytes, can be isolated for various purposes. The cells are harvested at an appropriate stage of development, which may be determined based on the expression of markers and phenotypic characteristics of the desired cell type. Cultures may be empirically tested by immunostaining or gene expression for the presence of the markers of interest, by morphological determination, *etc.* The cells are optionally enriched before or after the positive selection step by drug selection, panning, density gradient centrifugation, *etc.* In another example, a negative selection is performed, where the selection is based on expression of one or more of markers found on human ES cells, fibroblasts, neural cells, epithelial cells, and the like. Selection may utilize panning methods, magnetic particle selection, particle sorter selection, and the like.

[0014] Various somatic cells find use as a source of hiPSCs; of particular interest are adipose-derived stem cells, fibroblasts, keratinocytes, peripheral blood cells, and the like. The use of hiPSCs from individuals of varying genotypes, particularly genotypes potentially associated with neurologic and psychiatric disorders are of particular interest. The hiPSCs are dissociated as single cells, aggregated in spheroids of specific cell numbers and then grown in suspension; then induced to a neural fate by inhibition of BMP and TGF β pathways. The spheroids are then moved to medium in the presence of FGF2 and EGF and are patterned with Wnt pathway inhibitors or retinoic acid, as well as activators of the sonic hedgehog pathway. To promote differentiation, the spheroids are changed to medium comprising PDGF-AA, IGF-1, HGF, insulin, BDNF, NT3, cAMP, T3, and biotin. After such culture, the spheroids can be maintained for extended periods of time in neural medium containing insulin, ascorbic acid, cAMP, T3, and biotin in the absence of growth factors, e.g. for periods of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months or longer.

[0015] These and other objects, advantages, and features of the disclosure will become apparent to those persons skilled in the art upon reading the details of the subject methods and compositions as more fully described below.