CLAIMS

1. A vascular plant based cellulose scaffold for in-vitro production of neural stem cells.

2. The scaffold of claim 1, wherein the scaffold is obtained by decellularizing a vascular plant or a part thereof, or wherein the scaffold supports the growth of the neural stem cells, or wherein the vascular plant is asparagus or celery.

3. The scaffold of claim 1, wherein the scaffold is coated with a biomolecule, a synthetic biomolecule, a ligand, a protein, an amino acid, an antibody, an extracellular matrix, fibrin, laminin, collagen, fibronectin, poly-l-lysine, a small molecule, a hydrogel, or a Matrigel, or wherein the scaffold is coated with Poly-L-Ornithine (PLO).

4. The scaffold of claim 1, wherein the scaffold has a porous structure comprising a plurality of vascular bundles with varying diameters and a plurality of parenchymal cells:

wherein the vascular bundles are interspersed between the parenchymal cells; or

wherein the vascular bundles are unevenly spaced apart from each other and traverse the length of the scaffold, or

wherein the parenchymal cells form the surface of the scaffold, or

wherein the porous structure of the scaffold promotes attachment, proliferation or differentiation of the neural stem cells, or

wherein the porous structure of the scaffold allows the neural stem cells to maintain its differentiation ability, or

wherein the porous structure of the scaffold promotes differentiation of the neural stem cells into neurons, dopaminergic neurons, motor neurons, astrocytes, oligodendrocytes or a combination thereof, or

wherein the porous structure of the scaffold promotes differentiation of the neural stem cells into neurons and astrocytes, or

wherein the porous structure of the scaffold promotes increased production of the neural stem cells by enabling nutrient exchange, oxygen exchange and removal of waste within the scaffold.

5. The scaffold of claim 4, wherein the poly-L-ornithine coating increases production of the neural stem cells by promoting filopodia formation.

6. The scaffold of clam 1, wherein porosity of the scaffold ranges from 10% to 95%; or

wherein the scaffold has an elastic modulus in the range of 1 kPa to 1000 kPa; or

wherein the scaffold is biocompatible; or

wherein the scaffold is biodegradable; or

wherein the scaffold promotes three-dimensional growth of the neural stem cells as neurospheres on the scaffold; or

wherein the scaffold promotes the cells to grow neurite-like processes; or

wherein the scaffold promotes growth of multiple layers of the neural stem cells; or

wherein the scaffold promotes production of neural cell proteins.

7. The scaffold of claim 1, wherein the surface of the scaffold is modified by a chemical treatment or a physical treatment:

wherein the modification enhances attachment of the neural stem cells to the surface of the scaffold, or

wherein the surface of the scaffold is treated by functionalizing the surface, gamma irradiation, radical treatment, oxidative treatment, or a combination thereof, or

wherein the surface of the scaffold is treated by functionalization achieved by providing a functional group that creates a charge on the surface of the scaffold, wherein the functional group is a primary amine, a tertiary amine, a quaternary compound, an alcoholic group, a carboxylic acid group, an aldehyde group, a sulfonyl group or a combination thereof.

8. The scaffold of any one of claims 1-7, comprising a plurality of linear oriented channels, wherein the scaffold promotes growth of neural cells or endogenous neural cells in the plurality of linear oriented channels, wherein the scaffold promotes growth and/or regeneration of neuronal sensory tracts.

9. A vascular plant based scaffold according to any one of claims 1-8 for use in:

regenerative medicine or production of biopharmaceuticals; or

neural tissue engineering; or

treatment of neural disorders, defects or injuries; or

in-vitro drug production or in-vitro drug testing; or

in-vitro production of neural cell proteins or in-vitro harvesting of neural cell proteins; or

production of neural transplant or neural cell banks; or

production of a neural construct for in-vitro disease modeling; or

treatment of spinal cord injury, or in promoting hindlimb motor recovery and/or neural tissue repair in a subject with spinal cord injury.

10. A method of in-vitro production of neural stem cells:

a) seeding a culture medium comprising the neural stem cells in a container on a vascular plant based cellulose scaffold according to any one of claims 1 to 8; and

b) allowing the neural stem cells to attach and grow on the cellulose scaffold for at least 48 hours.

11. The method of claim 10, wherein the culture medium is infused with nutrients and growth factors to facilitate or increase attachment and growth of the neural stem cells to the scaffold; or

wherein the culture medium is DMEM MEM/F12, Aplha MEM, Knockout DMEM/F12, FBS, FCS, Goat Serum, or Horse Serum; or

wherein the culture medium is A:1X KnockOut DMEM/F-12 supplemented with 2% B27 (cat. 17504044 ThermoFisher), 2mM GlutaMAX-I (ThermoFisher),

B: serum free medium (1X KnockOut D-MEM/F-12 with 2% StemPro Neural Supplement ThermoFisher A1050801), 20 ng/mL bFGF, 20 ng/mL EGF, or 2mM GlutaMAX-I; or

wherein the method is carried out at a temperature ranging from 32 ºC to 42 ºC; or

wherein the method is carried out at a pH ranging from 6.8 to 7.2; or

wherein the container is a flask, a culture vessel, a bioreactor, a petri dish, a multi-well plate or a glass chamber.

12. The method of claim 10 or 11, wherein the method further comprises a step of coating the container prior to step a) with a natural biomolecule, a synthetic biomolecule, a ligand, a protein, an amino acid, an antibody, an extracellular matrix, fibrin, poly-L-ornithine, laminin, collagen, fibronectin, poly-l-lysine, a small molecule, a hydrogel, a Matrigel or a combination thereof.

13. The method of claim 12, wherein the container is coated with poly-L-ornithine and laminin.

14. The method of claim 10 or 11, further comprising a pre-processing step prior to step a), wherein the scaffold is pre-processed by:

sterilizing the scaffold with 70% ethanol;

washing the scaffold with a saline solution, a detergent solution, a physiological buffer or a salt solution; optionally wherein the washing step comprises an incubation step in which the scaffold is incubated in the salt solution overnight; or

pre-treating the surface of the scaffold; optionally:

wherein the surface is pre-treated by treating the surface chemically, treating the surface physically, functionalizing the surface, succinylating the surface or a combination thereof, optionally wherein the pre-treatment modifies the surface of the scaffold and enhances production of cells by increasing attachment to the surface of the scaffold.

15. The method of claim 14, wherein the surface is pre-treated by treating with a biomolecule, a synthetic biomolecule, a ligand, a protein, an amino acid, an antibody, an extracellular matrix, fibrin, laminin, collagen, fibronectin, poly-l-lysine, a small molecule, a hydrogel, a matrigel or a pharmaceutically acceptable compound; or wherein the surface is pre-treated by treating with a

synthetic biomolecule such as poly-L-ornithine, wherein the poly-L-ornithine coating increases production of the neural stem cells by promoting filopodia formation.

16. The method of claim 14, wherein the surface is functionalized by providing a functional group that creates a charge on the surface of the scaffold; wherein the functional group is a primary amine, a tertiary amine, a quaternary compound, an alcoholic group, carboxylic acid, aldehyde, sulfonyl or a combination thereof.

17. The method of claim 10, further comprising a sterilization step prior to step a), wherein the scaffold is sterilized for a pre-determined period of time, wherein the sterilization step is carried out by autoclaving, treatment with 70% ethanol, gamma irradiation, or treatment with ethylene oxide, optionally wherein the sterilization step is carried out for 20 minutes to several days.

18. The method of claim 10, wherein the production of neural stem cells is increased by modulating stiffness of the scaffold; or

wherein the production of neural stem cells is increased modulating anisotropy of the scaffold; or

wherein the neural stem cells differentiate to neurons, astrocytes, oligodendrocytes, or a combination thereof after attaching to the scaffold; optionally wherein the neural stem cell differentiation is determined by an immunostaining assay, cell imaging, confocal microscopy, flow cytometry, or electrophysiology; or

wherein the method further comprises an incubation step wherein the scaffold and the neural stem cells are incubated at a temperature ranging from 30ºC to 45ºC, optionally wherein the scaffold and the neural stem cells are incubated with CO_2; optionally wherein the scaffold and the neural stem cells are incubated at 37ºC with 5% CO_2.

19. The method of claim 14, further comprising a quantification step to quantify the production of the neural stem cells, wherein the quantification step is carried out by a biomolecule quantification assay, cell quantification assay, cell viability assay, image analysis, immunohistochemistry or electrophysiology.

20. The method of claim 19, wherein the quantification step is followed by an optimization step to optimize the growth conditions in the container, to optimize the quantity of scaffold, to optimize the size of the scaffold, to optimize the structure of the scaffold or a combination thereof.