CLAIMS

1. An *in vitro* method of generating a neuro-muscular junction cell culture model, comprising:

   a) cultivating a first cell culture comprising pluripotent stem cells (PSCs), for at least 3 days, in a first culture medium, comprising:
   - a WNT pathway-activator, and
   - FGF,

   b) replacing the first culture medium by a second culture medium, comprising:
   - a BMP pathway inhibitor, and (i) a TGFβ pathway inhibitor or (ii) GDF11,
   - Sonic Hedgehog (SHH) smoothened agonist (SAG) and/or retinoic acid (RA),
   - a WNT pathway-activator, and
   - FGF, HGF, and/or IGF1, and

   cultivating the cells for at least 3 days,

   thereby generating a cell culture model comprising segregated neural and mesodermal progenitor cells, and/or differentiated muscle cells with innervating motor neurons, forming neuromuscular junctions.

2. The *in vitro* method according to claim 1, wherein the cell culture model is a two-dimensional (2D), self-organizing neuro-muscular junction cell culture model.

3. The *in vitro* method according to claims 1 or 2, wherein the method further comprises step c):

   replacing the at least second culture medium by a culture medium comprising:
   - HGF and/or IGF1, and optionally a gamma-secretase inhibitor (e.g., DAPT), and

   cultivating the cells for at least 4 days, and optionally replacing the culture medium by a medium suitable for maintenance of the cell culture model,

   preferably wherein step b) further comprises replacing after 2-5 days the second culture medium by a third culture medium comprising retinoic acid and/or SAG, and optionally HGF and/or IGF1, and cultivating the cells for 2-5 days.

4. The *in vitro* method according to any one of claims 1-3, wherein the method comprises the steps of:

   a) Cultivating a first cell culture comprising pluripotent stem cells (PSCs; preferably iPS cells, more preferably iPSCs of a patient), for at least 3 days, in a first culture medium, comprising:
   - a WNT pathway-activator, and
   - FGF,

   b) replacing the first culture medium by a second culture medium, comprising one or more of the group comprising a WNT pathway-activator, an FGF, SHH smoothened agonist (SAG), a BMP pathway inhibitor, and either (i) a TGFβ pathway inhibitor and retinoic acid (RA), or (ii) GDF11, and cultivating the cells for 2-4 days,

c) replacing the second culture medium by a third culture medium comprising retinoic acid, SAG, HGF and IGF1, and cultivating the cells for 2-5 days, wherein on the first day a Rho-associated protein kinase inhibitor (ROCKi) is added to the culture medium for 6-24 hours,

d) replacing the third culture medium by a fourth culture medium comprising HGF and IGF1, and optionally a gamma-secretase inhibitor, and cultivating the cells for 1-5 days,

e) replacing the fourth culture medium by a fifth culture medium comprising HGF and IGF1, and cultivating the cells for at least 11 days,

f) replacing the fifth culture medium by a sixth culture medium comprising a ROCK inhibitor, ITS (Insulin, Transferrin, Selenium), HGF and IGF1, and cultivating the cells for 1-4 days, and

g) replacing the sixth culture medium by a seventh culture medium supplemented with ITS and cultivating the cells for at least one day.

5. The *in vitro* method according to any one of claims 1-4, wherein after 3 days of total culture duration the generation of brachial neurons and optionally cervical and/or thoracic neurons, is supported by adding a BMP pathway inhibitor, a TGFβ pathway inhibitor and optionally retinoic acid (RA), and/or wherein after 3 days of total culture duration the generation of lumbar neurons is supported by adding a BMP pathway inhibitor and GDF11.

6. The *in vitro* method according to any one of claims 1-5, wherein after 6 days of total culture duration at least a fraction of the neural progenitor cells expresses SOX2 and at least a fraction of the mesodermal progenitor cells expresses PAX3, and wherein the cells segregate into neural and mesodermal progenitor clusters, and/or

   wherein after 20 days of total culture duration at least a fraction of the neural progenitor cells are brachial neurons and optionally cervical and/or thoracic neurons, expressing one or more of HOXC6, HOXC5, HOXC8, HOXC9, ISL1, CHAT and TUBB3, and/or

   wherein after 20 days of total culture duration, at least a fraction of the neural progenitor cells are lumbar neurons expressing one or more of HOXC10, HOXC9, ISL1, CHAT and TUBB3, and/or

   after 20 days of total culture duration at least a fraction of the neural progenitor cells expresses one or more of the factors of TUBB3, ISL1 and CHAT, and the muscle progenitor cells express one or more of the factors of DESMIN, MYOD and MYF5, and/or

   wherein after 50 days of total culture duration at least a fraction of the neurons expresses TUBB3 and at least a fraction of the muscle cells expresses MSKF, DESMIN, and/or TITIN.

7. The *in vitro* method according to any one of claims 1-6, wherein the first cell culture comprises pluripotent stem cells of a patient, and wherein the patient has been diagnosed with and/or is suffering from a myopathy, motor-neuron disease, an auto-immune

neuromuscular disease, or other disease affecting the muscular or neuromuscular system of said patient.

8. An *in vitro* cell culture model produced according to the method of any one of claims 1-7, comprising (i) segregated neural and mesodermal progenitor cells, (ii) segregated partially differentiated neural and myoblast cells, (iii) brachial, and optionally cervical and/or thoracic, motor neurons, or (iv) lumbar motor neurons, wherein the models of (iii) and/or (iv) comprise bundles of muscle fibers surrounded by innervating motor neurons forming neuromuscular junctions.

9. An *in vitro* cell culture model, comprising (i) segregated neural and mesodermal progenitor cells, (ii) segregated partially differentiated neural and myoblast cells, and/or (iii) bundles of muscle fibers surrounded by innervating motor neurons, forming neuromuscular junctions.

10. An *in vitro* cell culture model according to claim 9, comprising segregated neural and mesodermal progenitor cells, wherein at least a fraction of the neural progenitor cells expresses SOX2, and at least a fraction of the mesodermal progenitor cells expresses PAX3, and wherein at least a fraction of the cells segregates into neural and mesodermal progenitor clusters.

11. An *in vitro* cell culture model according to claim 9, comprising segregated partially differentiated neural and myoblast cells, wherein at least a fraction of the neural cells expresses TUBB3, and at least a fraction of myoblast cells expresses DESMIN and form bundles of fibers that are surrounded by and/or at least partially interwoven with axonal structures of neural cells.

12. The *in vitro* cell culture model according to claim 11, wherein at least a fraction of the bundles of fibers has a length of over 50μm, and at least a fraction of the axonal structures of neural cells has a length of over 20μm.

13. An *in vitro* cell culture model according to claim9, comprising neuro-muscular junctions, wherein the model comprises bundles of muscle fibers surrounded by innervating motor neurons forming functional neuromuscular junctions, wherein at least a fraction of the neurons express TUBB3, and at least a fraction of the muscle cells expresses MSKF and/or MYF5.

14. The *in vitro* cell culture model according to claim 13, wherein at least a fraction of the motor neurons form axons of 50-600 μm length and at least a fraction of the muscle fibers has a length of 100-3000 μm.

15. An *in vitro* cell culture model according to claim 9, comprising differentiated brachial, and optionally cervical and/or thoracic, motor neurons, comprising neuro-muscular junctions, wherein at least a fraction of the motor neurons expresses one or more of HOXC6, HOXC5, HOXC8, ISL1, CHAT and TUBB3, and/or comprising differentiated lumbar motor neurons comprising neuro-muscular junctions, wherein at least a fraction of the motor neurons expresses one or more of HOXC10, HOXC9, ISL1, CHAT, ISL1 and TUBB3.

16. The *in vitro* cell culture model according to claim 9, wherein the cell culture model is a two-dimensional (2D), self-organizing neuro-muscular junction cell culture model.

17. The *in vitro* cell culture model according to claim 9, wherein the model is established from pluripotent stem cells of a patient, and wherein the patient has been diagnosed with and/or is suffering from myopathy, motor-neuron diseases, an auto-immune neuromuscular disease, or a disease affecting the muscular or neuromuscular system of said patient.

18. A method for drug screening, toxicity screening and/or for studying a disease, comprising the *in vitro* cell culture model according to any one of claims 8-17.

19. A kit comprising a supplement-cocktail and/or cell culture medium, comprising:

    - a WNT pathway-activator,
    - an FGF,
    - a BMP pathway inhibitor,
    - (i) a TGFβ pathway inhibitor or (ii) GDF11,
    - A Sonic Hedgehog (SHH) smoothened agonist (SAG) and/or retinoic acid (RA), and
    - HGF and/or IGF1,
    - optionally comprising a gamma-secretase inhibitor.