# Claims

1. A serum-free method for producing engineered skeletal muscle tissue from pluripotent stem cells, comprising the steps of

   (i) inducing mesoderm differentiation of the pluripotent stem cells by culturing pluripotent stem cells in a basal medium comprising an effective amount of (a) FGF2, (b) a GSK3 inhibitor, (c) a SMAD inhibitor, and (d) a serum-free additive comprising transferrin, insulin, progesterone, putrescine and selenium or a bioavailable salt thereof;

   (ii) inducing myogenic specification by culturing the cells obtained in step (i) in a basal medium comprising an effective amount of (a) a gamma-secretase/NOTCH inhibitor, (b) FGF2, and (c) a serum-free additive as in (i), followed by
   continuing the cultivation in the medium with the addition of an effective amount of (d) HGF, followed by
   culturing the cells in a basal medium comprising an effective amount of (a) a gamma secretase/NOTCH inhibitor, (b) HGF, (c) a serum-free additive as in (i), and (d) knockout serum replacement (KSR);

   (iii) expanding and maturing the cells into skeletal myoblasts and satellite cells by culturing the cells obtained in step (ii) in a basal medium comprising an effective amount of (a) HGF, (b) a serum-free additive as in (i), and (c) knockout serum replacement (KSR);

   (iv) maturing the cells into skeletal myotubes and satellite cells by culturing the cells obtained in step (iii), which are dispersed in an extracellular matrix, under mechanical stimulation in a basal medium, comprising an effective amount of (a) a serum-free additive as in step (i), and (b) an additional serum-free additive comprising albumin, transferrin, ethanolamine, selenium or a bioavailable salt thereof, L-carnitine, fatty acid additive, and triiodo-L-thyronine (T3);

   thereby producing engineered skeletal muscle tissue, wherein the skeletal muscle tissue generates a contraction force of at least 0.6 millinewtons (mN) upon a stimulus of 100 Hz.

2. The method of claim 1, wherein in step (iv), the mechanical stimulation is a static tension, a dynamic stimulation, or an auxotonic stimulation.

3. The method of any one of claims 1-2, comprising, prior to step (i), a seeding step, wherein the pluripotent stem cells are seeded in a stem cell medium in the presence of a ROCK inhibitor.

4. The method of any one of claims 1-3, wherein after step (iii), the skeletal myoblasts and satellite cells are seeded into an engineered form in an additional step prior to step (iv) in the presence of one or more components of an extracellular matrix in a master mix.

5. The method of claim 4, wherein the engineered form has the form of a ring, ribbon, strand, patch, pouch, or cylinder, wherein optionally individual skeletal muscle tissues are fused.

6. The method of any one of claims 1-5, wherein the method does not comprise a differentiation- or maturation-related transgene.

7. The method of any one of claims 1-6, wherein the basal medium in step (iv) comprises an effective amount of creatine and/or triiodo-L-thyronine (T3).

8. The method of any one of claims 1-7, wherein the skeletal muscle tissue has a contraction speed of at least 3 mN/sec upon a stimulation of 100 Hz.

9. The method of any one of claims 1-8, wherein the skeletal muscle tissue has a relaxation speed of at least 0.5 mN/sec upon termination of a stimulation of 100Hz.

10. An engineered skeletal muscle tissue, having multinuclear mature skeletal muscle fibers with satellite cells, and having no blood supply and/or no central nervous system control, wherein the skeletal muscle tissue is serum-free and/or does not comprise a differentiation- or maturation-related transgene, wherein the skeletal muscle tissue generates a contraction force of at least 0.6 millinewtons (mN) upon a stimulus of 100 Hz.

11. The engineered skeletal muscle tissue of claim 10, wherein the skeletal muscle tissue does not comprise a myogenic transgene.

12. The engineered skeletal muscle tissue of claim 11, wherein the skeletal muscle tissue does not comprise the Pax7 or MyoD transgene.

13. The engineered skeletal muscle tissue of any of claims 10-12, wherein the skeletal muscle tissue has a contraction speed of at least 3 mN/sec upon a stimulation of 100 Hz.

14. The engineered skeletal muscle tissue of any of claims 10-13, wherein the skeletal muscle tissue has a relaxation speed of at least 0.5 mN/sec upon termination of a stimulation of 100Hz.

15. A skeletal muscle tissue according to any of claims 10-14 for use in medicine.

16. An *in vitro* method for testing the efficacy of a drug candidate on a skeletal muscle tissue, comprising the steps of
    (a) providing a skeletal muscle tissue according to any of claims 10-14,
    (b) optionally inflicting damage on the skeletal muscle tissue, and
    (c) contacting the skeletal muscle tissue of step (a) or (b) with a drug candidate.

17. An *in vitro* method for testing the toxicity of a substance on a skeletal muscle tissue, comprising the steps of
    (a) providing a skeletal muscle tissue according to any of claims 10-14,
    (b) contacting the skeletal muscle tissue from step (a) with a substance to be tested.

18. An *in vitro* method for testing the effect of nutrients and dietary supplements on skeletal muscle tissue performance, comprising the steps of
    (a) providing a skeletal muscle tissue according to any of claims 10-14,
    (b) contacting the skeletal muscle tissue from step (a) with a nutrient or dietary supplement to be tested.