Claims

1. A method for producing bioengineered heart muscle from pluripotent stem cells, comprising the steps of
   (i) cultivating pluripotent stem cells in a basal medium comprising an effective amount of (a) BMP4, Activin A, FGF2, a GSK3-inhibitor, and (b) a serum-free supplement resulting in a final concentration of 0.5-50 mg/ml albumin, 1-100 µg/ml transferrin, 0.1-10 µg/ml ethanol amine, 0.003-0.3 µg/ml sodium selenite, 0.4-40 µg/ml L-Carnitine HCl, 0.1-10 µg/ml Hydrocortisone, 0.05-5 µl/ml Fatty acid supplement, 0.0001-0.1 µg/ml triodo-L-thyronine (T3), thereby inducing mesoderm differentiation of said pluripotent stem cells;
   (ii) cultivating the cells obtained in step (i) in a basal medium comprising an effective amount of an inhibitor of the Wnt-signaling pathway and a serum-free supplement as in (i), thereby inducing cardiac differentiation of the cells; and
   (iii) cultivating the cells obtained in step (ii) in a basal medium comprising an effective amount of a serum-free supplement as in (i), under mechanical stimulation, thereby promoting cardiac maturation.

2. The method of claim 1, wherein the pluripotent stem cells are selected from embryonic stem cells, induced pluripotent stem cells, and parthenogenetic stem cells; and/or wherein the pluripotent stem cells are pluripotent stem cells of primate origin, preferably human pluripotent stem cells.

3. The method of any one of claims 1-2, wherein the basal medium in step (i) comprises
   1-20 ng/ml BMP4, preferably about 5 ng/ml BMP4; and
   0.1-10 ng/ml FGF2, preferably about 5 ng/ml FGF2; and
   1-20 ng/ml Activin A, preferably about 9 ng/ml Activin A.

4. The method of any one of claims 1-3, wherein the GSK3-inhibitor in the basal medium of step (i) is selected from the group consisting of CHIR99021, CHIR98014, SB216763, TWS119, Tideglusib, SB415286, and LY2090314; preferably wherein the basal medium in step (i) comprises 0.1-10 µM CHIR99021.

5. The method of claim 1, wherein the inhibitor of the Wnt-signaling pathway in the basal medium of step (ii) is selected from the group consisting of IWP4, IWP2, IWR-1, IWP1, IWP3, IWR-2, IWR-3, IWR-4, IWR-5, XAV939, DKK1, quercetin, ICG-001, pyrvinium, CCT031374, iCRT-3,5,14, CPG049090, NC043; preferably wherein the basal medium of step (ii) comprises 0.1-10 µM IWP4.

6. The method of any one of claims 1-5, wherein the basal medium of step (iii)
   a) further comprises 0.1-10 ng/ml TGFβ1, most preferably about 1 ng/ml TGFβ1; and/or
   b) does not comprise an effective amount of FGF2; and/or
   c) comprises 0.5-3 mM Ca^{2+}, most preferably about 1.2 mM Ca^{2+}.

7. The method of any one of claims 1-6, wherein the mechanical stimulation in step (iii) is dynamic mechanical stimulation or static stretch, preferably wherein the mechanical stimulation in step (iii) is dynamic mechanical stimulation.

8. The method of any one of claims 1-7, wherein the basal medium of step (i), (ii) and/or (iii) comprises 10-1000 µM, preferably 50-400 µM, more preferably 100-300 µM, even more preferably 150-250 µM, and most preferably about 200 µM of ascorbic acid or a salt thereof.

9. The method of any one of claims 1-8, wherein the serum-free supplement in step (i), (ii) and/or (iii) comprises 0.1-10 % B27 or B27 minus insulin, preferably 0.5-8 %, more preferably 1-6 %, even more preferably 1.5-4%, and most preferably about 2% B27 or B27 minus insulin.

10. The method of any one of claims 1-9, wherein the basal medium used in step (i), (ii) and/or (iii) is DMEM/F12, StemPro, Iscove's medium, αMEM, DMEM, and RPMI, preferably wherein the basal medium used in step (i), (ii) and/or (iii) is RPMI supplemented with pyruvate.

11. The method of claim 1, comprising prior to step (i) a seeding step, wherein said pluripotent stem cells are seeded in a ratio of (2.5-6 x 10^6 cells / 1 mg collagen) / 1 ml medium in a suitable mould; preferably wherein the seeding step is carried out 18-30 h prior to step (i).

12. The method of claim 11, wherein the medium used in the seeding step further comprises a ROCK-inhibitor, preferably selected from Y27632, H-1152P, Thiazovivin, Fasudil, Hydroxyfasudil, GSK429286A, and RKI-1447, more preferably wherein the medium used in the seeding step comprises 1-50 μM Y27632.