SUPPLEMENTAL METHODS AND FOGURES

Supplemental Methods

Cell Culture

The human induced pluripotent stem (hiPS) cell line WTC11 was kindly provided by Dr. Bruce Conklin (through a Material Transfer Agreement with Gladstone Institute of Cardiovascular Disease, UCSF). The hiPS cells were maintained in mTeSR 1 medium (Stem cell Technologies) supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin at 37°C in a humidified incubator with room air and 5% CO_2. The hiPS cells were continuously passaged at 60-70% confluency into 6 well plates and routinely checked for mycoplasma contamination. For the first 24h after passaging, culture medium was supplemented with 2 µM of Thiazovivin (Tocris 3845). The plates were coated with 1 mL of Growth Factor Reduced Matrigel (Corning 356231) diluted 1:80 in DMEM/F12 media prior to use.

hiPS cells were differentiated into cardiomyocytes using published methods routinely used in our laboratory.[20] Briefly, hiPSC cardiac differentiation was initiated at 90-95% confluency. Differentiation was achieved through addition of CDM3 (RPMI 1640 (ThermoFisher 11875093) supplemented with 213 µg/mL ascorbic acid (Wako Chemicals 321-44823), 500 µg/mL albumin (ScienCell OsrHSA), and 100 U/mL penicillin and 100 µg/mL streptomycin) supplemented with 3 µM CHIR 99021 (Tocris 4423) for two days, followed by CDM3 supplemented with 2 µM Wnt-C59 (Tocris 5148) for another two days. Media was continuously changed with CDM3 ever two days afterwards.

Bioreactor design

A custom mold for the bioreactor was designed in SOLIDWORKS and manufactured out of polyoxymethylene using a computer numerical control (CNC) milling machine (Haas OM 2). Sylgard 184 Polydimethylsiloxane (PDMS) was mixed in a 10:1 ratio with Sylgard 184 curing agent, and poured into the custom mold. The molds were placed under vacuum to remove bubbles, and left to cure in the oven

at 60°C overnight. The subsequently formed reactors were washed and plasma bonded to a glass slide (75 x 50 mm).

Bioreactor cultivation of cardiac constructs

hiPS-CMs were dissociated from monolayers using 0.2% collagenase II (Worthington LS004177) in HBSS at 12-17 days after the start of differentiation. Prior to construct seeding, the construct formation wells were coated with 4% Pluronic F127 (Sigma P2443) for 30 minutes, washed, and then allowed to air dry for 30 minutes. Approximately 25 x 10^6 hiPS-CMs/mL were encapsulated in collagen-fibrinogen hydrogel consisting of 1.5 mg/mL of rat tail collagen type I (Corning CB354249), 4 mg/mL bovine fibrinogen (Sigma F8630), and 10 units/mL thrombin (Sigma T7513). 120 µL of the cell-hydrogel solution were placed into each well of the bioreactor to form a cardiac construct around the pillars. The constructs were cultured in RPMI 1640 medium (Thermofisher 11875) supplemented with 2% B27 (Thermofisher 17504044), 213 µg/mL ascorbic acid (Wako Chemicals 321-44823), 100 U/mL penicillin and 100 µg/mL streptomycin. Aprotinin (33 µg/mL, Sigma A3428) was added to the media for the first 7 days of culture. Culture media was changed every two days, and constructs were analyzed after two weeks of culture. Relatively immature constructs were analyzed after three days of culture.

Western blot preparation

For western blot analysis, 3-4 constructs per group were pooled together, flash-frozen in liquid nitrogen, and then transferred to a -80°C freezer until they were ready for protein isolation. Constructs were homogenized using TissueRuptor II (Qiagen) in Tissue Protein Extraction Reagent (Thermofisher 78510) supplemented with 1X Protease/Phosphatase Inhibitor Cocktail (Cell Signaling Technology 5872S). The homogenized constructs were then placed on a vortex mixer at 4°C for 2 hours. Protein concentrations were then determined using a Pierce BCA protein assay kit (Thermofisher 23225), and 30 µg of total protein were added to each lane.