Direct Hydrogel Encapsulation of Pluripotent Stem Cells Enables Ontomimetic Differentiation and Growth of Engineered Human Heart Tissues

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Supplementary Material

Fig. S1. Cluster and single encapsulated hiPSCs grew and proliferated in PEG-fibrinogen hydrogels. (A) Higher numbers of cluster hiPSCs proliferated on tissue edges, while encapsulated single hiPSCs grew independent of tissue location. (B) Initially, cells grew and occupied void spaces within the pre-existing tissue footprint, followed by a tissue growth beyond the original microisland boundaries which resulted in a lateral surface area increase of 20.1 ± 8.0 % for IMR90-1 hiPSC encapsulations and 25.5 ± 8.0 % for 19-9-11 hiPSC encapsulation early during differentiation (day 5, n = 3-4 tissues). (C) Z-stack slice of day 10 PCNA-stained cluster encapsulated tissue edge indicated a high number of proliferating cells on the edge of the tissue. Cell nuclei were counterstained with DAPI. Star is on side of tissue edge. (D) High magnification phase contrast images of cluster encapsulated hiPSCs on day 5 and day 30 revealed differences in tissue density, leading to a dense tissue ring around the microisland perimeter. White arrows indicate locations with higher cell density.

Fig. S2. Contraction properties of 3D-dhECTs. (A) Frequency of spontaneous contraction increased in cluster encapsulation cardiac tissues over time, ranging from 0.60 ± 0.21 Hz (Early) to 0.92 ± 0.16 Hz (Intermediate) to 1.37 ± 0.04 Hz (Late). n = 3 independent differentiation batches. Mean ± s.d. During long-term culture, frequency of contraction increased to 2.2 ± 0.1 Hz (day 50) followed by a subsequent decrease to 1.78 ± 0.16 Hz (day 60) and 1.35 ± 0.05 Hz (day 90). n = 2 3D-dhECTs. Mean ± s.d. (B) Microislands formed using cluster encapsulated hiPSCs displayed higher contractility (vs. single hiPSC microislands) based on video analysis of spontaneously contracting cardiac tissues on day 14 of differentiation (see corresponding Movies S3 and S4). Y-axis represents the change in transmitted light intensity obtained from bright field microscopy as an index of contractility. Valleys correspond to the relaxed state, and peaks indicate contraction. AU (arbitrary units).

Fig. S3. (A) Isotype control and (B) cTnT (cardiac marker) and P4HB (fibroblast marker) stained 3D-dhECT cells resulted in 72.5 ± 3.2% CMs (n = 5 tissues) and 5.24 ± 2.72 % fibroblast positive cells (n = 3 tissues).

Fig. S4. MEA recordings of 3D-dhECTs responding to exogenous pacing and beta-adrenergic agonist and antagonist drug treatment. (A) Day 20 3D-dhECTs responded to exogenous pacing up to 3 Hz (at 37ºC). (B) Representative traces of 3D-dhECT spontaneous activity showing changes in field potential duration of baseline, following 1 μM isoproterenol addition, subsequent 1 μM propranolol addition, and after washout.

Fig. S5. Development of discrete sarcomere structure during ontomimetic cardiac differentiation. (A-C) 2D FFT analysis of selected αSA-stained 3D-dhECTs provided evidence to an increase in sarcomere alignment and definition over time, represented by the transition from no visible rings (day 20) to visible, full rings (day 30) to more defined, aligned (direction of sarcomeres) rings (day 124). (D) Sarcomere distance of day 124 3D-dhECT CMs was quantified by generated intensity plots (increased gray scale represents existing sarcomere).

Fig. S6. CMs within the dense 3D-dhECTs continue to develop during extended *in vitro* culture. (A) Day 52 dissociated 3D-dhECT CMs exhibited an elongated morphology and stained positive for Caveolin 3, providing a first indication of T-tubule development. (B, C) In long-term cultured cardiac tissues, an area of increased cell density was observed on tissue edges as well as tissue outgrowth onto the acrylated glass; this tissue outgrowth contained CMs that exhibited a less mature phenotype with less defined sarcomeres and Cx43 distributed in a punctate pattern along the borders of the cells. Images are focused to visualize the bottom 2D tissue outgrowth (B) and overall tissue structure (C). Arrows indicates tissue edge. (D) Example subregion of TEM image (day 124) and plot of grayscale intensity profile for identification and counting of Z-lines (Z), neighboring I-bands (I), and H-zone (H).

Fig. S7. Initial cell-seeding density can be varied to produce contracting 3D-dhECTs. (A-D) Original cell-seeding density (60 million/ml) and (E-H) 50% of original cell-seeding density (30 million/ml) was compared during early stages of tissue formation. Increase in cell number (darkening effect) over time was observed for both cell-seeding densities.

Movies S1-S6

Movie S1. Z-stack (8 frames, 35 μm total thickness) of cluster encapsulated hiPSCs shows proliferating cells (PCNA positive, red) on day 10 of differentiation. Tissue was counterstained with DAPI (blue).

Movie S2. Representative video showing a spontaneously contracting 3D-dhECT microisland on day 8 of differentiation using the 19-9-11 hiPSC line.

Movie S3. Representative video showing contraction of 3D-dhECT microisland formed using cluster encapsulated hiPSCs (day 14 of cardiac differentiation).

Movie S4. Representative video showing contraction of 3D-dhECT microisland formed using single encapsulated hiPSCs (day 14 of cardiac differentiation).

Movie S5. Representative video showing contraction of a 3D-dhECT macrotissue on day 20 of differentiation.

Movie S6. Representative video showing contraction of a 3D-dhECT microsphere on day 20 of differentiation.

Supplementary Table 1 References

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