Electronic Supplementary Information (Table S1 and Figures S1-S4)

One-Step Microfluidic Generation of Pre-Hatching Embryo-Like Core-Shell Microcapsules for Miniaturized 3D Culture of Pluripotent Stem Cells

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Movies S1-S3 are available as three other separate files.

Table S1: List of primers for qRT-PCR studies

Oct-4	F	5'-GAAGCCCTCCCTACAGCAGA-3'
	R	5'-CAGAGCAGTGACGGGAACAG-3'
Sox-2	F	5'-GCATGTCCTACTCGCAGCAG-3
	R	5'-GCTGATCATGTCCCG GAGGT-3'
Nanog	F	5'-CCCCACAAGCCTTGGAATTA-3'
	R	5'-CTCAAATCCCAGCAACCACA-3'
Klf2	F	5'-CTGCTGGAGGCCAAGCCCAA-3'
	R	5'AGGTGGTCGGACCTGGAGAA-3'
Brachyury (T)	F	5'-CTCTAATGTCCTCCCTTGTTGCC-3'
	R	5'-TGCAGATTGTCTTTGGCTACTTTG-3'
Nkx2.5	F	5'-GATGGGAAAGCTCCCACTATG-3'
	R	5'-GAGACACCAGGCTACGTCAATA-3'
cTnT	F	5'-GAAGTTCGACCTGCAGGAAA-3'
	R	5'-TTCCCACGAGTTTTGGAGAC-3'
GAPDH	F	5'-CTCTGGCTCAGAGGGTTTGG-3'
	R	5'-ACAGAAACCAGTGGGCTTTGA -3'

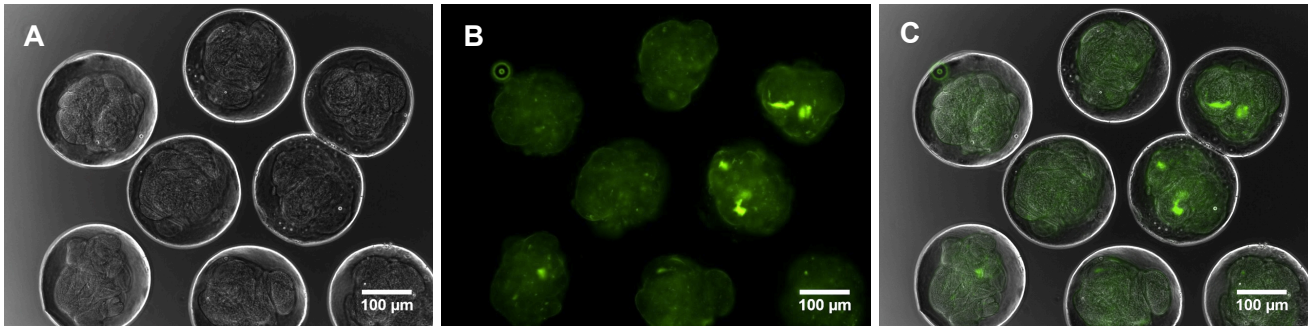

Figure S1. Microcapsules with a collagen core and alginate hydrogel shell generated using the non-planar microfluidic device in one step: (A), typical phase contrast image showing the core-shell morphology of microcapsules with collagen being labeled with FITC in the core; (B), the corresponding fluorescence image showing collagen in the core of microcapsules in panel A; and (C), a merged view of the images in panels A and B showing the colocation of the fluorescence collagen and the visible core of microcapsules. For making microcapsules with the collagen core, the procedure was the same as that for making the microcapsules with a liquid core shown in Fig. 2B except that a core solution containing 6 mg ml^{-1} (2.6% of which was labeled with FITC for visualization using fluorescence microscopy), 0.225% cellulose, and 0.034 M mannitol was used, the experiments were done in 4 °C cold room, and the collagen was gelled at 37 °C for 45 min in the collecting tube before centrifuging to separate the microcapsules from oil into aqueous phase.

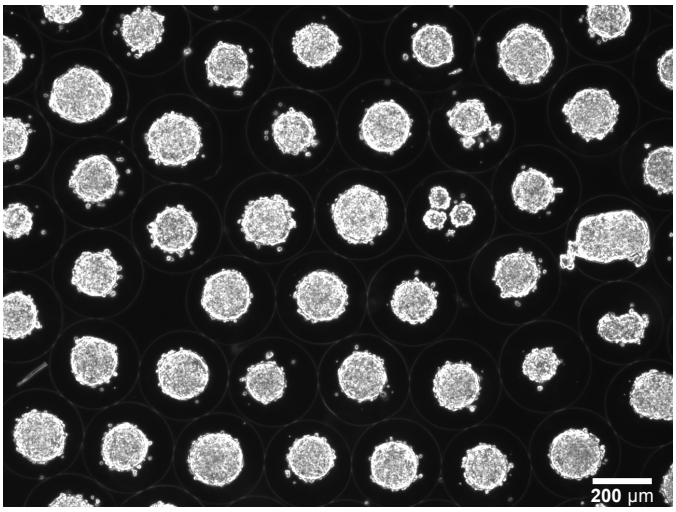

Figure S2. Low magnification image (4x) demonstrating ES cell aggregates of fairly uniform size formed in pre-hatching embryo-like core-shell microcapsules at day 7.

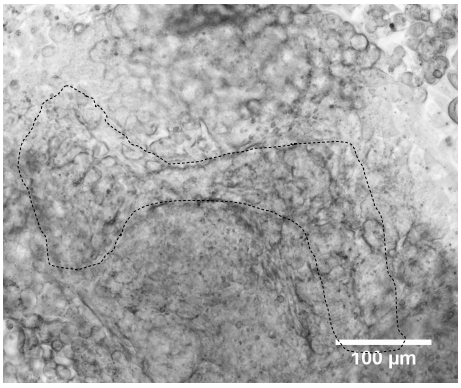

Figure S3. A typical image of the aggregated cells on day 12 of differentiation showing a beating focus (marked by dashed line, a corresponding Movie S3 is given in a separate file)

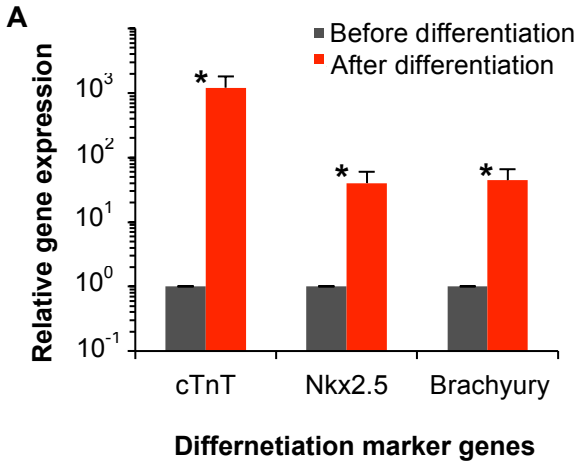

Figure S4. Data from qRT-PCR showing significantly higher expression of cardiac troponin T (cTnT), cardiac progenitor cell marker (Nkx2.5), and mesodermal cell marker (brachyury or T) in the differentiated cells compared to the aggregated ES cells before differentiation. *: Statistically significant ($p < 0.05$)