Electronic Supplementary Information (Table S1 and Figures S1-S5)

Coaxial electrospray of liquid core-hydrogel shell microcapsules for encapsulation and miniaturized 3D culture of pluripotent stem cells

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Movies S1 is available as a separate file.

Table S1. List of primers used 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'
SSEA-1	F	5’-GGAGGGAGCAGTGACGCTAAC-3’
	R	5’-GTATGGGAGGGCGATTCGA-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'
Nestin	F	5'-GGAGTGTCGCTTAGAGGTGC-3'
	R	5'-TCCAGAAAGCCAAGAGAAGC-3'
Sox-7	F	5'-ACCTTCAGGGGACAAGAGTTCG-3'
	R	5'-GTTTTTCTCAGGCAGCGTGTTC-3'
GAPDH	F	5'-CTCTGGCTCAGAGGGTTTGG-3'
	R	5'-ACAGAAACCAGTGGGCTTTGA -3'

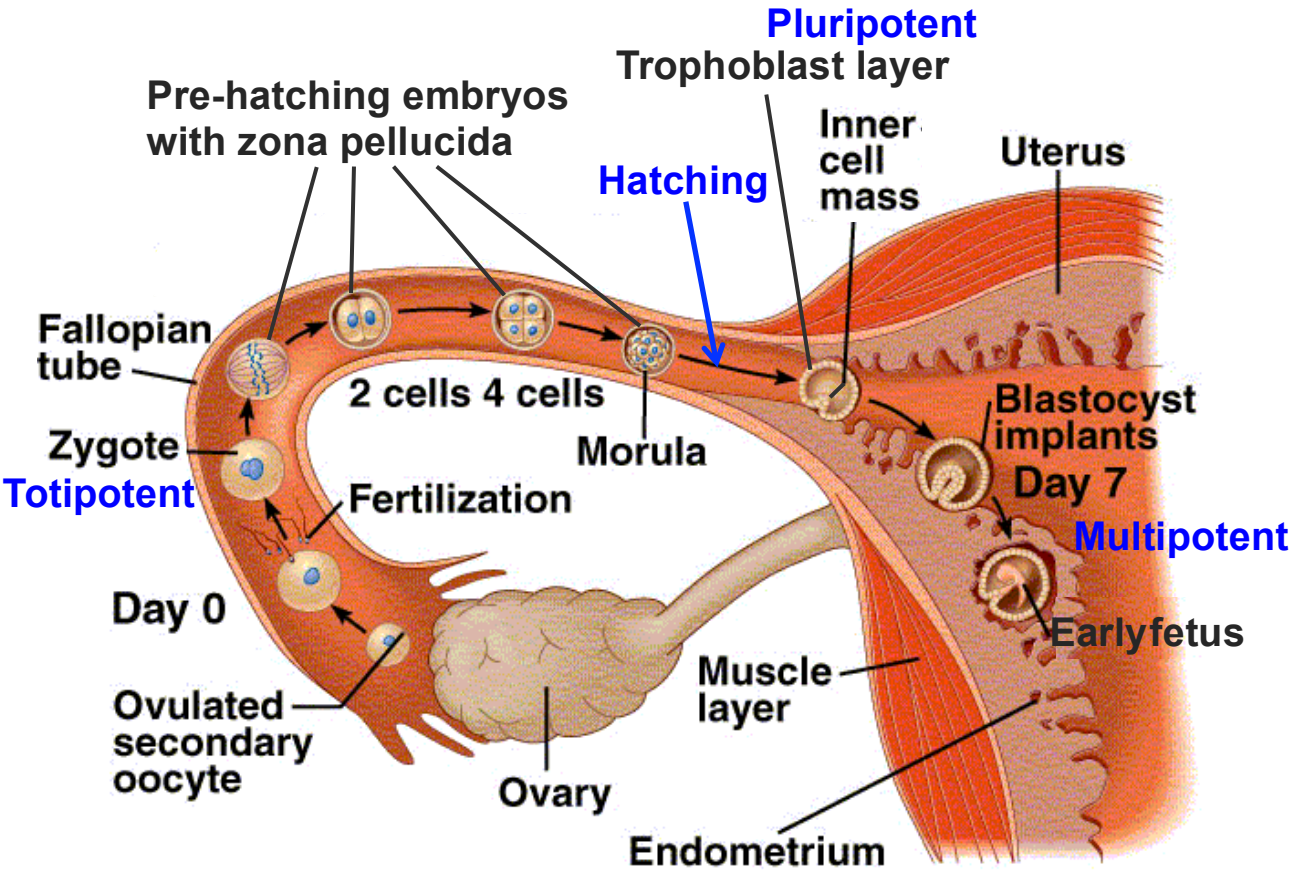

Figure S1. A schematic illustration of embryo development in the female reproductive system starting from the totipotent zygote (one-cell embryo stage): The zygote develops to 2-cell, 4-cell, 8-cell, and eventually morula (of many cells) stages within the zona pellucida, followed by the formation of trophoblast layer (underneath the inner wall of zona pellucida in early blastocyst) that allows hatching to release late blastocyst (i.e., what is within the trophoblast layer) out of the zona pellucida. The hatched embryo (i.e., late blastocyst) further implants into the inner layer (i.e., endometrium) of uterus wall, followed by further development of the trophoblast layer and inner cell mass (containing embryonic stem cells) into the placenta and early fetus, respectively. During the embryo development, the single totipotent cell gradually divides and differentiates into many pluripotent stem cells in the zona pellucida before hatching. With the formation of a trophoblast layer to allow hatching out of zona pellucida and implantation into endometrium, the pluripotent stem cells gradually divide and differentiate into lineage/organ specific multipotent stem cells. Therefore, we hypothesize that the zona pellucida plays a crucial role in maintaining "stemness" form the totipotent to pluripotent states before hatching. This figure is adapted from Reference 11 (R. Lewis, D. Gaffin, M. Hoefnagels, B. Parker, in *Life (5th Ed.)*. McGraw-Hill Higher Education: Columbus, OH, 2004).

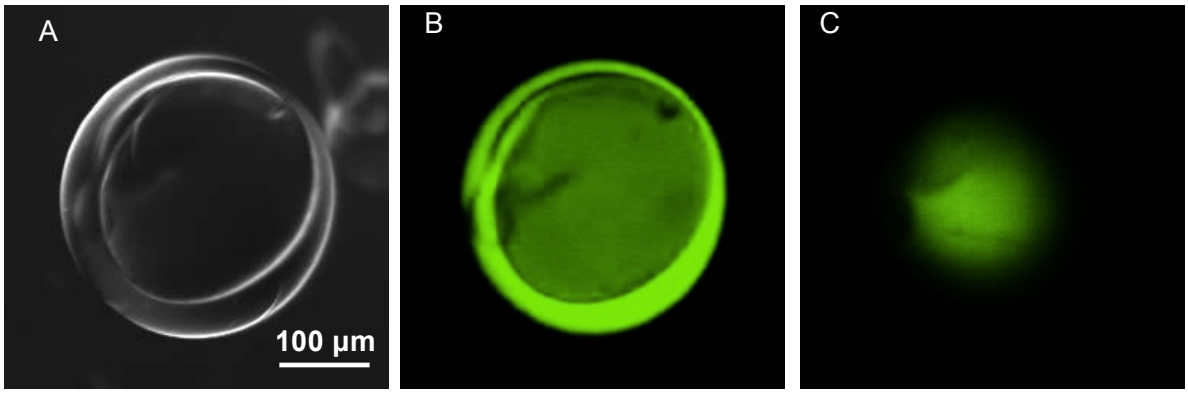

Figure S2. Core-shell architecture of microcapsules produced by coaxial spray of two aqueous fluids studied using confocal microscopy: (A) differential interference contrast (DIC) micrograph showing the overall morphology of a microcapsule, (B) fluorescence confocal micrograph of the microcapsule in a plane cutting through the middle of the microcapsule showing its core-shell morphology, and (C) fluorescence confocal micrograph of the microcapsule in a plane cutting through the top of the microcapsule in its shell. The microcapsule was made by adding 0.5% high molecular weight dextran (500 kD) labeled with FITC (a green fluorescence probe) in the shell fluid using the coaxial electrospray method with other conditions being the same as that used for making microcapsules shown in Fig. 1B. The background fluorescence in the core of the microcapsule in (B) probably was a result of leaking of the FITC-dextran from the shell into the core, as we did observe the decrease of fluorescence in the microcapsule shell in water with time.

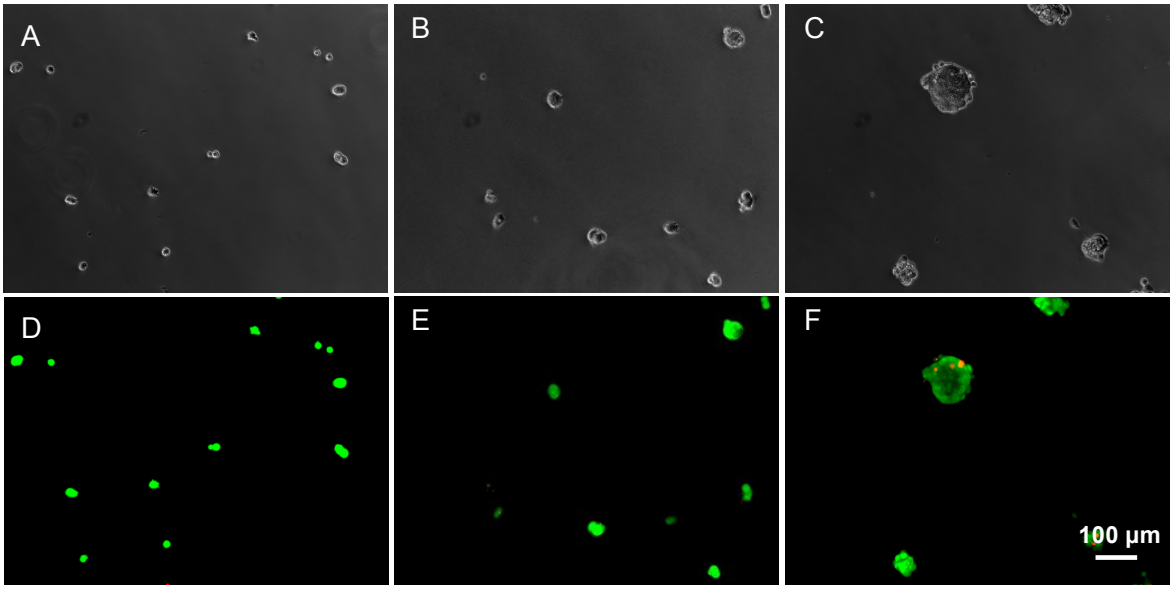

Figure S3. Survival and proliferation of ES cells under 2D culture: typical phase contrast (A-C) and fluorescence micrographs (D-F) showing the morphology of ES colony on 2D surface and the viability at 12 h (A and D), on day 2 (B and E), and on day 3 (C and F) in complete ES cell medium. The 2D colonies on day 3 are collected to determine the pluripotency or cultured in differentiation medium containing BMP4 and bFGF for further cardiac differentiation. According to the instructions from ATCC, the R1 ES cells should not be continuously cultured for more than 3 days on 2D substrate to better maintain their pluripotency.

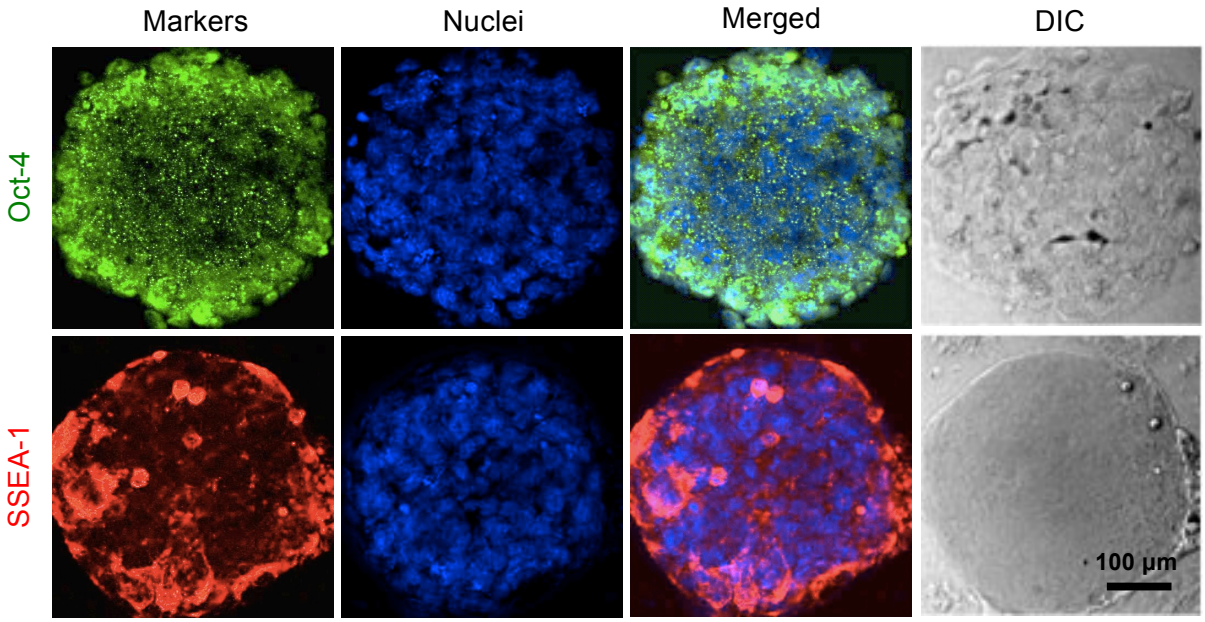

Figure S4. The pluripotency of ES cell aggregates formed in the miniaturized 3D liquid core of microcapsules with an alginate hydrogel shell: Immunohistochemical staining of Oct-4, SSEA-1, and cell nuclei together with differential interference contrast (DIC) micrographs showing high expression of the pluripotency marker proteins in the aggregated ES cells.

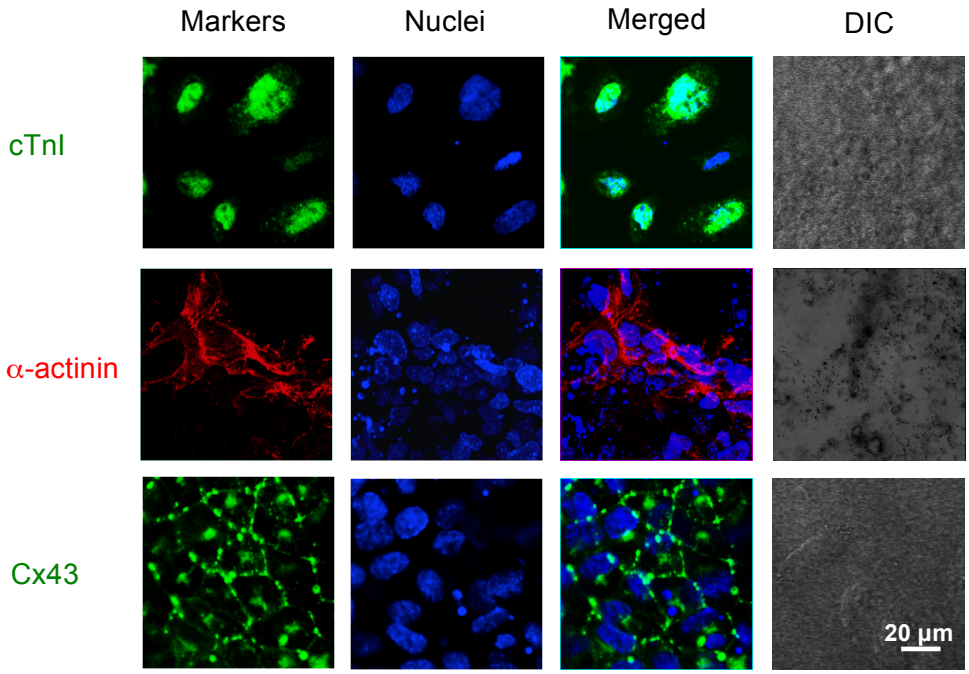

Figure S5. Cardiac differentiation of ES cell aggregates formed in the miniaturized 3D liquid core of microcapsules with an alginate hydrogel shell: Confocal fluorescence micrographs of immunohistochemical staining of the cTnI (top) together with two other proteins important for the proper function of cardiomyocytes: α-actinin (middle) in cardiac sarcomere and connexin 43 (Cx43, bottom) in gap junction between cardiomyocytes.