Supporting Information

A Biomimetic Core-Shell Platform for Miniaturized 3D Cell and Tissue Engineering

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Methods

Fabrication of non-planar microfluidic device: Silicon master with patterned microfluidic channels was prepared using a multilayer photolithography technique.[25,28] Briefly, photosensitive epoxy (SU-8 2025, MicroChem) was spun-coated onto a 4-inch silicon wafer (spread cycle-500 rpm at 100 rpm/sec with a total time of 10 sec; spin cycle-800/1750 rpm for 100/50 μm thickness at 300 rpm/sec for 30 sec). A 100 μm layer of photoresist was first coated and then soft-baked at 95 °C for 20 min, followed by exposure to UV light through the first shadow mask printed with the core channel. After a post-exposure baking at 95 °C for 10 min, an additional layer (50 μm) of photoresist was spun coated, soft baked, and exposed with a second shadow mask to pattern shell channel. The third layer for oil/extraction channel was similarly patterned. All three exposures were aligned using an EVG620 automated mask aligner. In the end, the pattern on the substrate was developed in SU-8 developer solution (MicroChem) for 15 min, rinsed with isopropyl alcohol, and dried using nitrogen gas. The mixture of cross-linker and PDMS pre-polymer (1:10) was then poured on the silicon substrate and cured at 65 °C for at least 3 h to form PDMS slab. Thereafter, the PDMS slab with micro-channels was lifted off. Two PDMS slabs with the same channel design were then plasma-treated for 30 s using a Harrick PDC-32G plasma cleaner at 18 W and 27 Pa, wetted with methanol (to prevented instant bonding), aligned and bonded together under microscope to produce the final microfluidic device. Assembled device was kept on a hotplate at 80 °C for ~10 min to evaporate

residual methanol and further kept at 65 °C for 2 days to make it sufficiently hydrophobic for experiments.

Animals: Peromyscus maniculatus bairdii (BW stock) deer mice were purchased from the *Peromyscus* Genetic Stock Center at the University of South Carolina, Columbia, SC and bred. CD1 mice for isolating mouse embryonic fibroblasts (MEFs) cells were purchased from Charles River. Both deer mice and CD1 mice were maintained on a 16-8 h light-dark cycle before the experiments. All procedures for animal use were approved by the Institutional Animal Care and Use Committee (IACUC) at The Ohio State University and every effort was made to minimize animal suffering.

Cell culture: R1 mouse ESCs from ATCC (Manassas, VA) were cultured in medium consisted of Knockout DMEM (Millipore, Billerica, MA) supplemented with 15% (v/v) Knockout Serum (Millipore), 4 mM L-glutamine (Sigma, St. Louis, MO), 100 μg ml^{-1} antibiotics (Invitrogen) and 1000 U ml^{-1} leukemia inhibitory factor (LIF) (Millipore), 10 μg ml^{-1} gentamicin (Sigma) and 0.1 mM mercaptoethanol (Sigma) on a gelatin coated tissue culture flasks at 37 °C in a humidified 5% CO_2 incubator. When reaching desired ~70% confluence, cells were detached using trypsin/EDTA (Invitrogen, Carlsbad, CA) and gently pipetted to break aggregates. Cells were centrifuged, re-suspended, and counted for further passaging or experimental use.

Isolation of preantral follicles: Preantral follicles (100-135 μm) were isolated using mechanical methods from ovaries of female deer mice of 12 to 16-week old as reported by us previously.[28,34] In brief, the ovaries were placed in 2 ml Leibovitz L-15 medium (Invitrogen) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Invitrogen) and 1% (v/v) penicillin–streptomycin. Preantral follicles were obtained by using two 30 G needles to mechanically break the extracellular matrix between follicles in the ovarian tissue and then immediately used for encapsulation. Encapsulated preantral follicles were co-cultured with MEFs in follicle culture medium consist of *α*-minimum essential medium-glutamax supplemented with 5% (v/v) heat-inactivated fetal bovine

serum (FBS) with 5 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml selenium, and 100 mIU/mL recombinant human follicle stimulating hormone (FSH).

Preparation of mouse embryonic fibroblasts (MEFs): MEFs were isolated by following a protocol reported elsewhere.[4] In brief, E13.5 mouse embryos were dissected and the brain, limbs, and internal organs were removed to ensure a pure fibroblastic population. Embryos were minced with a sterile razor blade into small pieces and they were placed in 37 °C in 5% CO_2 air for 10 min. The samples were then pipetted up and down using 1 ml pipette to further breakup the tissue, followed by culturing in 100 mm culture dish coated with 0.1% gelatin at 37 °C in 5% CO_2 air. MEFs were then expanded and frozen for future use. For experiments, MEFs (passage 3) at 2×10^4 cells/well were seeded on 96-well plates followed by treatment with 10 μg ml^{-1} mitomycin for 3 h. Afterwards, MEFs were washed twice with 1x PBS and 100 μl of follicle culture medium with encapsulated preantral follicle was added.

Supplementary references

[1] P. Agarwal, S. Zhao, P. Bielecki, W. Rao, J. K. Choi, Y. Zhao, J. Yu, W. Zhang, X. He, *Lab Chip* **2013**, *13*, 4525.
[2] J. K. Choi, P. Agarwal, H. Huang, S. Zhao, X. He, *Biomaterials* **2014**, *35*, 5122.
[3] J. K. Choi, P. Agarwal, X. He, *Tissue Eng Part A* **2013**, *19*, 2626.
[4] J. Xu, *Curr Protoc Mol Biol*, Wiley, **2005**, Vol. 70, p. 28.1.1.

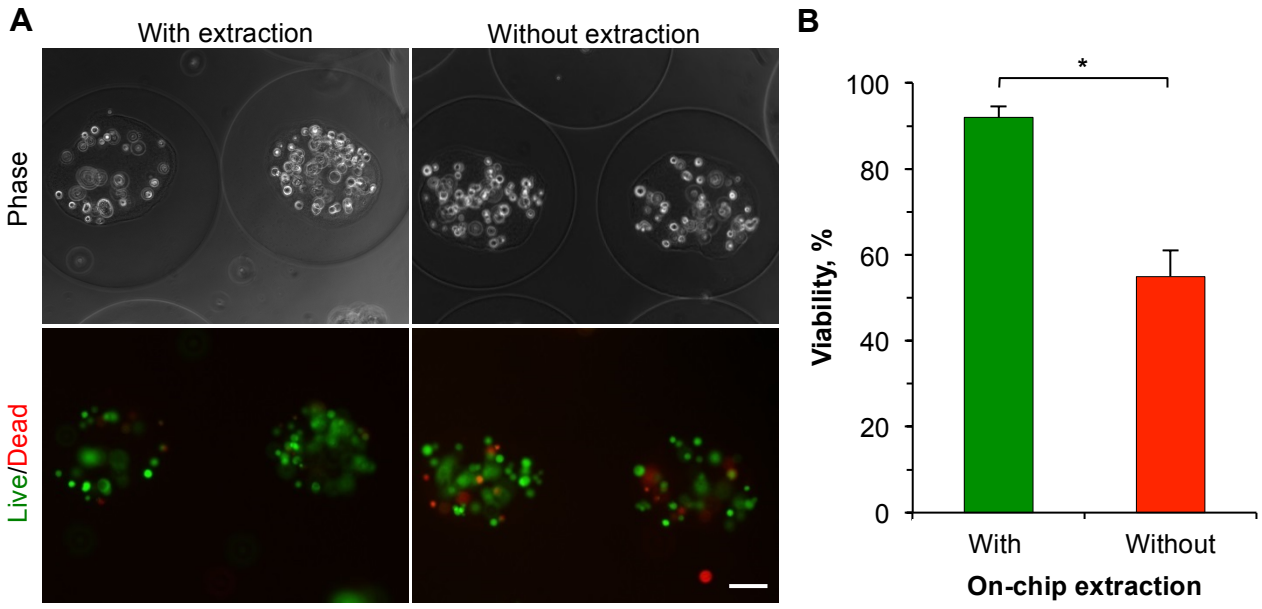

Figure S1. (A) Typical phase contrast and fluorescence images (live/dead viability staining) of encapsulated mouse embryonic stem cells (mESCs) in the microcapsules after collected with or without on-chip extraction. (B) Quantitative data of cell viability in microcapsules obtained with and without on-chip extraction. Scale bar: 100 μm. * denotes $p < 0.05$.

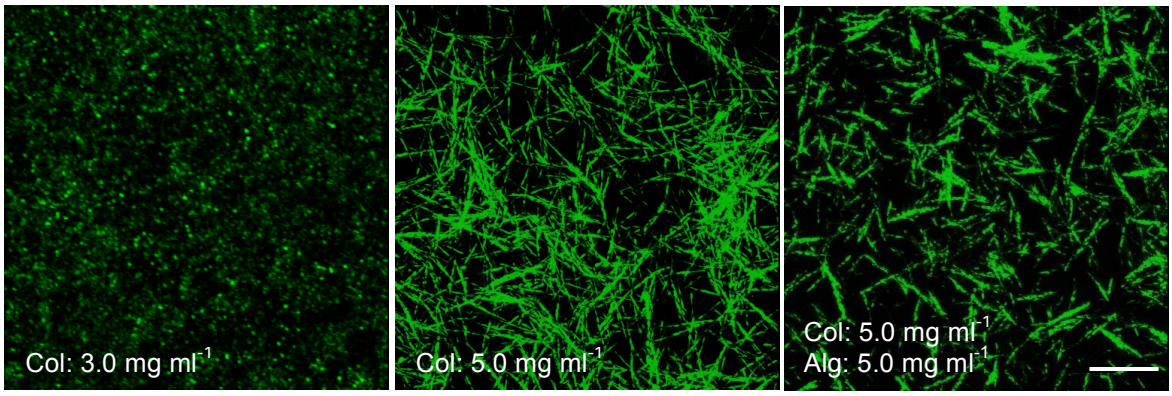

Figure S2. Confocal reflectance microscopy (CRM) images of fibers in the core ECM made of 3.0 mg ml^{-1} collagen, 5.0 mg ml^{-1} collagen, and a mixture of 5.0 mg ml^{-1} collagen and 5.0 mg ml^{-1} alginate. Col: collagen. Alg: alginate. Scale bar: 20 μm.

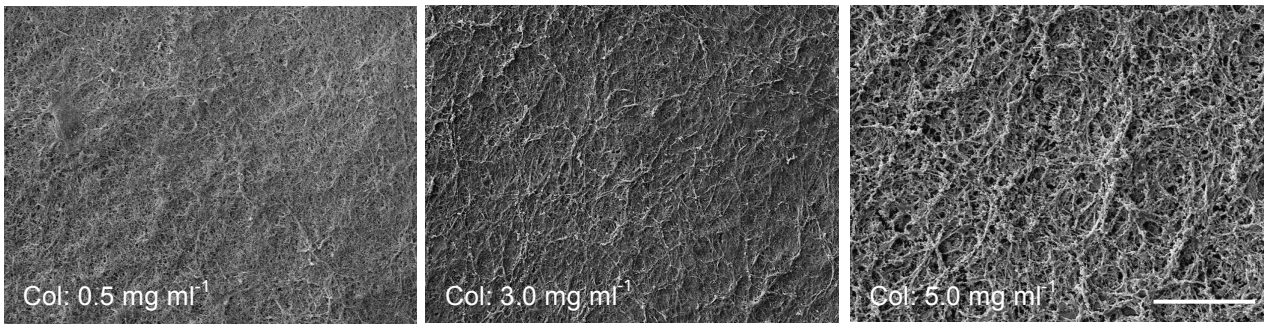

Figure S3. Scanning electron microscopy (SEM) images of fibers in 0.5 mg ml^{-1} collagen, 3.0 mg ml^{-1} collagen, and 5.0 mg ml^{-1} collagen. To take the SEM images, core-shell microcapsules with different ECMs were processed using a standard procedure by fixing with glutaraldehyde, dehydrating using a series of ethanol treatment, and chemical drying using hexamethyldisilazane (HMDS). Since glutaraldehyde could dissolve alginate, we did not perform SEM of ECM containing a mixture of 5.0 mg ml^{-1} collagen and 5.0 mg ml^{-1} alginate. Col: collagen. Scale bar: 20 μm.

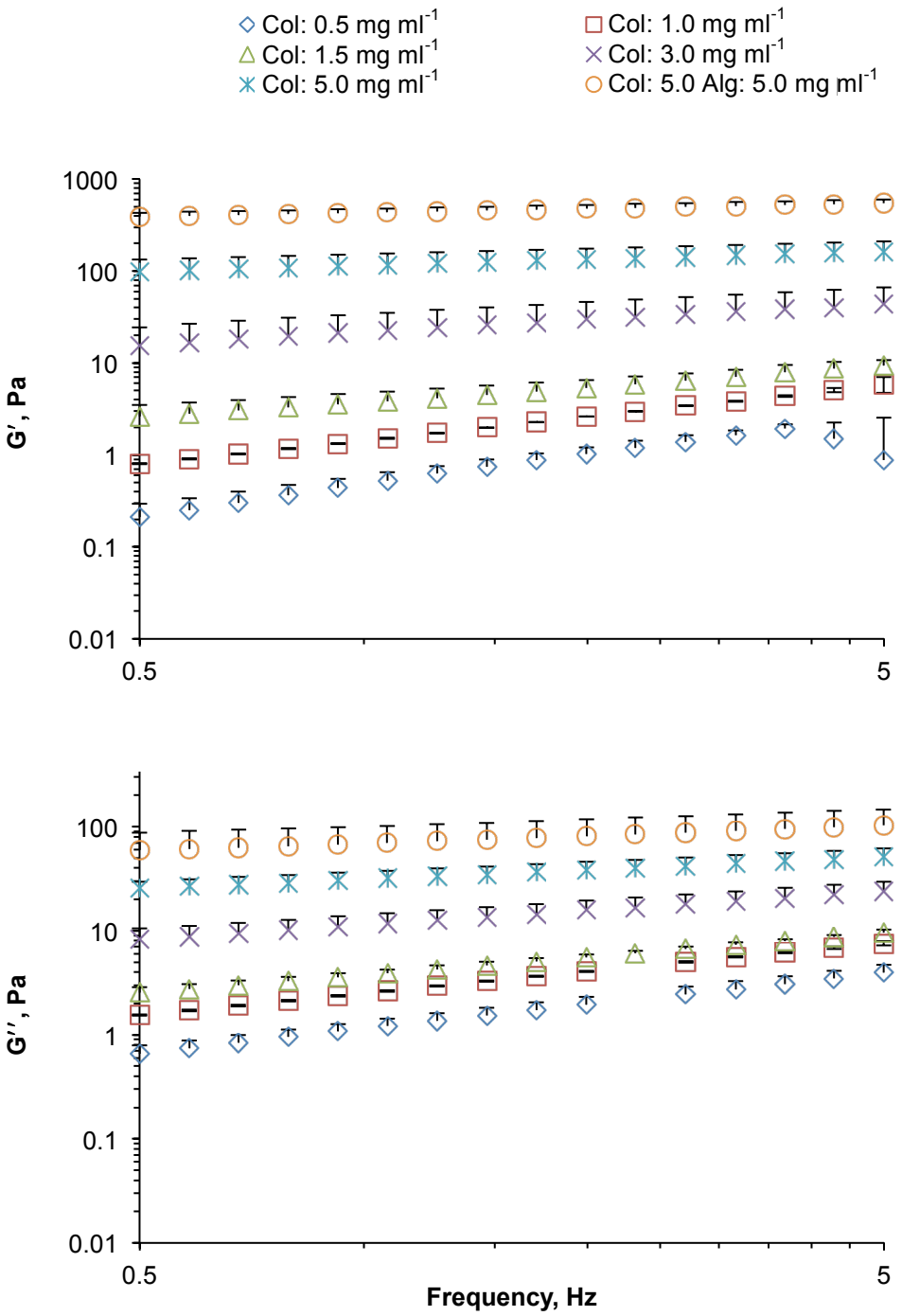

Figure S4. Quantitative data of storage (G′) and loss (G′′) modulus of different core ECMs used for encapsulating cells/tissues as a function of oscillation frequency. The data at 1 Hz were reported in Figure 2F for comparison. As the concentration of collagen increases and with the addition of alginate, the moduli increase. Col: collagen. Alg: alginate.

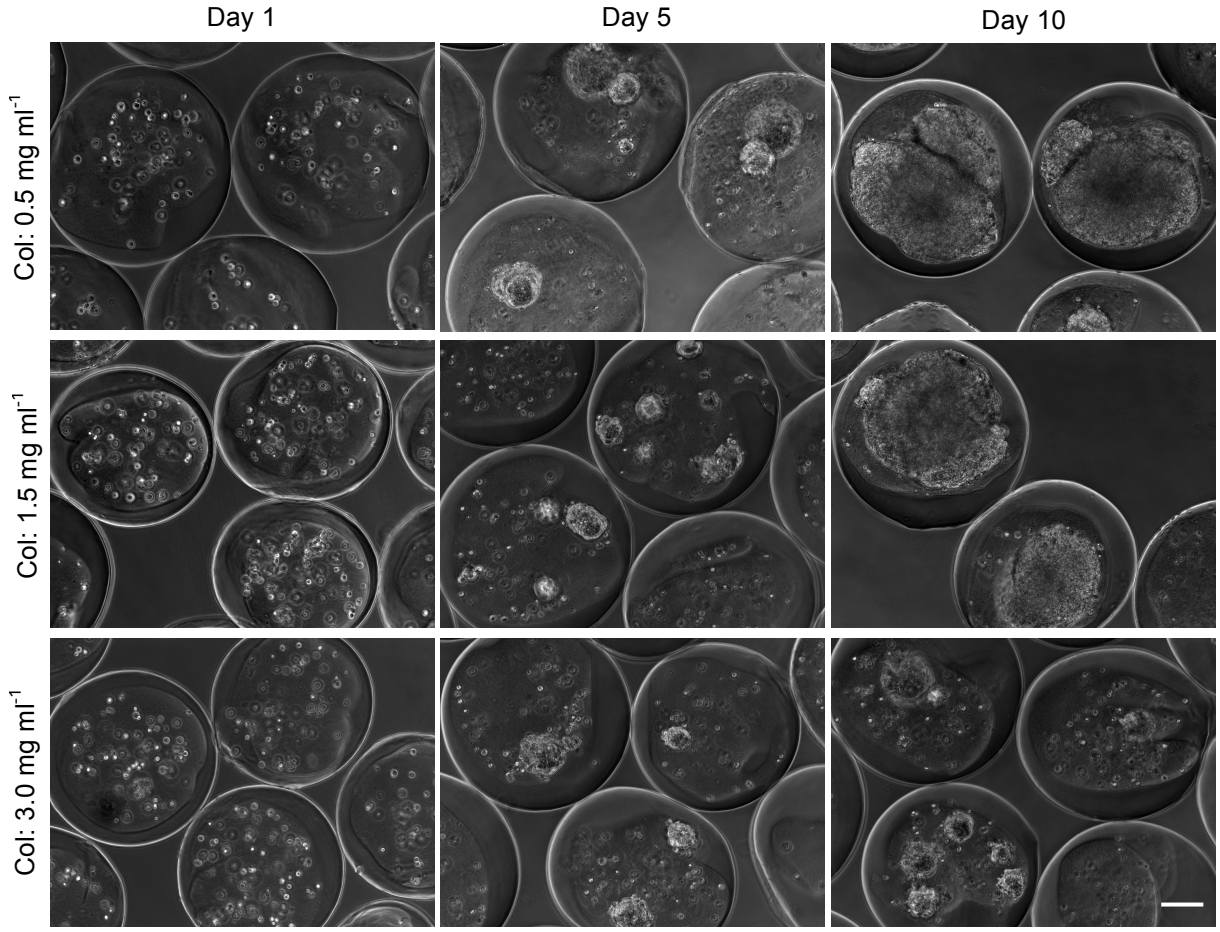

Figure S5. Typical phase images of encapsulated mouse embryonic stem cells (mESCs) on days 1, 5, and 10 showing their proliferation in 0.5, 1.5, and 3.0 mg ml^{-1} collagen core ECMs. As observed from the images, mESCs showed higher proliferations in the softer ECM made of 0.5 or 1.5 mg ml^{-1} collagen where they formed single massive aggregates compared to the small and multiple aggregates in the harder ECM made of 3.0 mg ml^{-1} collagen. Col: collagen. Scale bar: 100 μm.

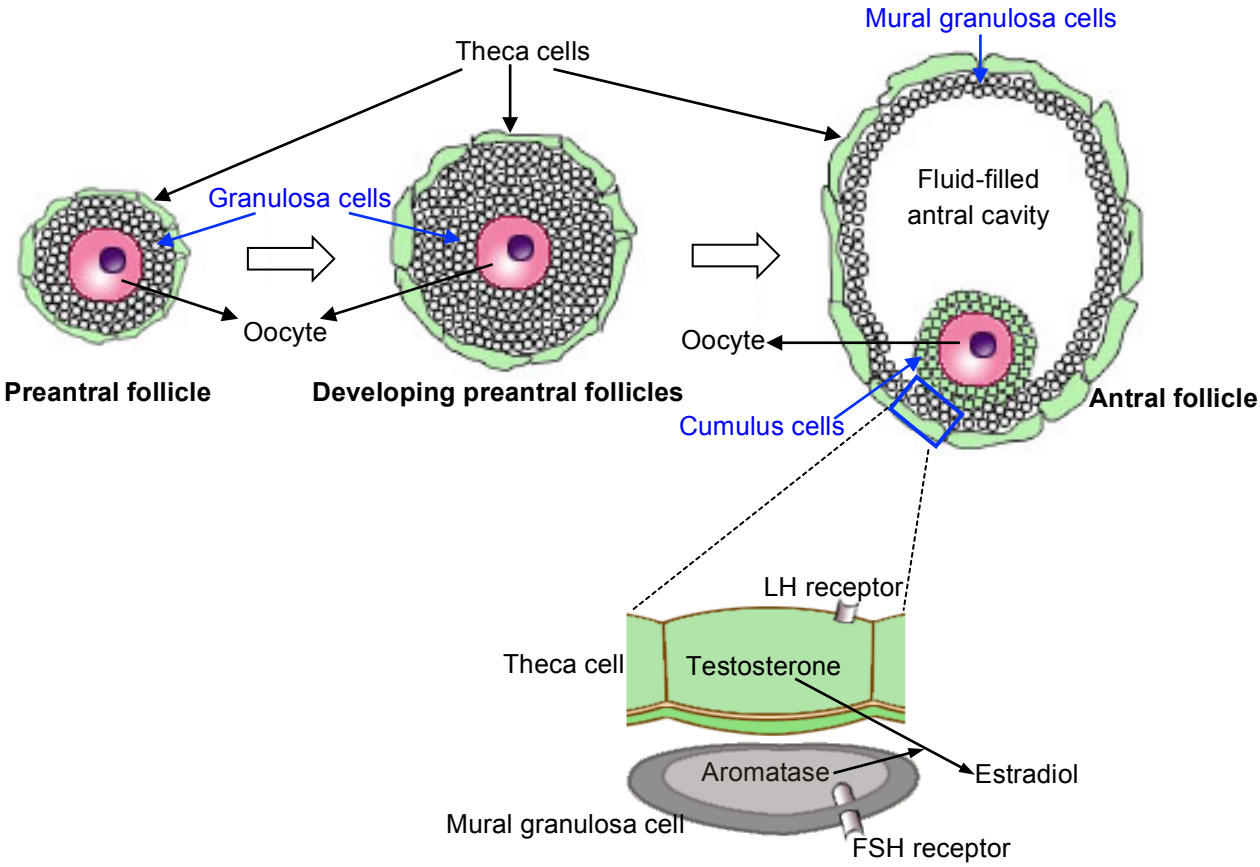

Figure S6. A schematic illustration of the anatomy and hormone (estradiol) secretion of ovarian follicles developing from the early secondary preantral to the antral stage. An early secondary preantral follicle consists of a single oocyte, few layers of granulosa cells, and an outer rim of theca cells. During development, granulosa cells proliferate to generate additional layers followed by differentiation into cumulus cells and mural granulosa cells. An antral follicle is characterized by the presence of a cumulus-oocyte complex (COC) inside a fluid-filled antral cavity (also called antrum). Estradiol production requires the cooperative function between theca and mural granulosa cells. Mural granulosa cells of an antral follicle secrete aromatase that converts testosterone secreted by theca cells into estradiol. LH: Luteinizing hormone. FSH: Follicle stimulation hormone.

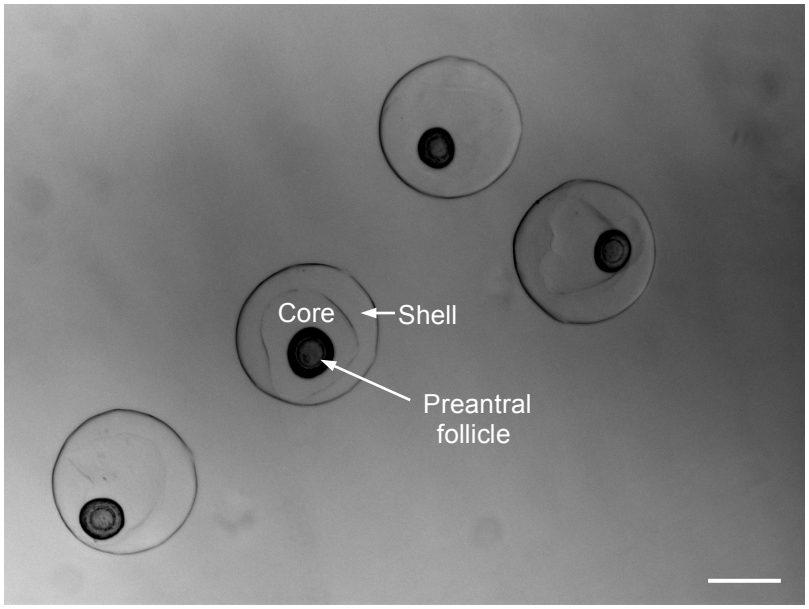

Figure S7. Typical bright field image of deer mouse preantral follicles encapsulated in 5.0 mg ml^{-1} collagen core ECM of microcapsules with a 2% alginate hydrogel shell on day 1. Scale bar: 200 μm.

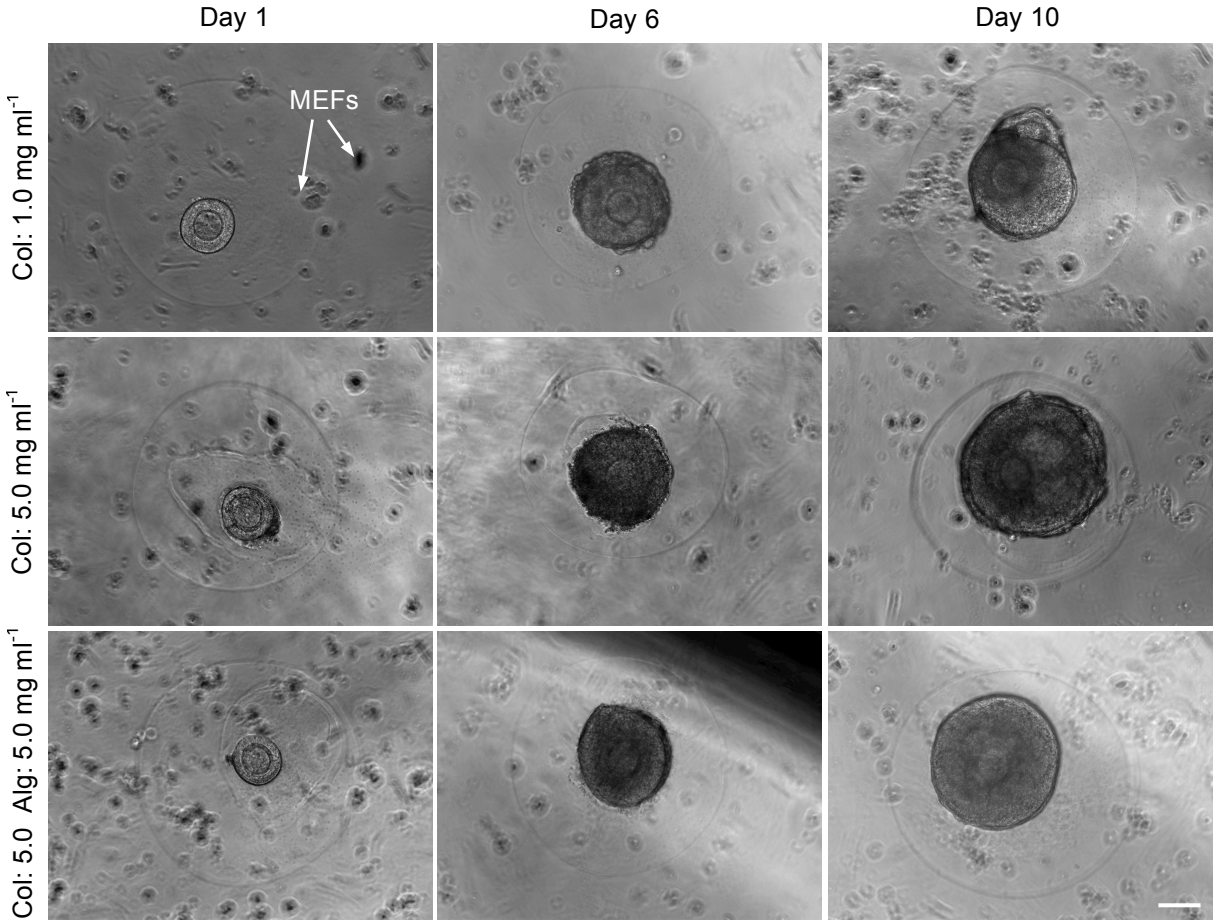

Figure S8. Typical phase images of encapsulated deer mouse preantral follicles on days 1, 6, and 10 showing their proliferation and development in core ECMs made of 1.0 mg ml^{-1} collagen, 5.0 mg ml^{-1} collagen, and a mixture of 5.0 mg ml^{-1} collagen and 5.0 mg ml^{-1} alginate. Encapsulated preantral follicles were cultured on feeder layer of mouse embryonic fibroblasts (MEFs) at a cell density of 2×10^4 in 96- well plate. Col: collagen. Alg: alginate. Scale bar: 100 µm.

Table S1. *In vitro* culture of preantral follicles in different core ECMs of microcapsules with a shell made of 2% alginate hydrogel.

Core ECM	# of preantral follicles	# (%) of antral follicles	# (%) of MII oocyte	# of two-cell embryo
Col: 1.0 mg ml^{-1}	38	1 (2.6)	0 (0)	0
Col: 5.0 mg ml^{-1}	45	11 (24.4)	5 (45.5)	1[a]
Col: 5.0 mg ml^{-1} Alg: 5.0 mg ml^{-1}	37	4 (10.8)	0 (0)	0

Col: collagen. Alg: alginate.

[a] 3 MII oocytes were used to obtain embryo by parthenogenetic activation.

Table S2. List of primers used for quantitative RT-PCR studies of the expression of four pluripotency marker genes (Oct-4, Sox2, Nanog, and Klf2) together with GAPDH as the housekeeping gene.

Oct-4	F	5'-GAAGCCCTCCCTACAGCAGA-3'
	R	5'-CAGAGCAGTGACGGGAACAG-3'
Sox2	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'
GAPDH	F	5'-CTCTGGCTCAGAGGGTTTGG-3'
	R	5'-ACAGAAACCAGTGGGCTTTGA -3'