Expansion of Breast Cancer Stem Cells with Fibrous Scaffolds

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Supplement Material

Materials and Methods

Immunofluorescence staining and western blot were performed as described previously.[1] Antibodies used were rabbit anti-vimentin (D21H3, Cell Signaling Technology; dilution 1:100), rabbit anti-*E*-cadherin (24E10, Cell Signaling Technology; dilution 1:100), and Alexa Fluor 488-conjugated goat anti-rabbit IgG (Molecular Probe; dilution 1:200).

Supplementary Table and Figure

Table S1. List of primers

Gene Name	Forward sequence 5'-3'	Reverse sequence 3'-5'
SOX2	GCTTTTGTTCGATCCCAACTTTC	ATGGATTCTCGGCAGACTGATTC
OCT3/4	CGAAAGAGAAAGCGAACCAGTATC	AGAACCACACTCGGACCACATC
SOX4	GCATGATGATAGCATATGTGTTCAGGT	CGGCATATTGCACAGGATGGA
ITGA6	CAAATGCAGGCACTCAGGTTC	GCATCAAGATCCCAGCGAGA
ITGA5	GTCAGCAGCTCCTATATGTGACCAGA	CCCTCGGGATCCAACTCCAG
SNAIL	GCCTAGCGAGTGGTTCTTCTG	CTGCTGGAAGGTAAACTCTGGATT
SLUG	GCGAACTGGACACACATACAGTGAT	GTGGAATGGAGCAGCGGTAGT
ZEB1	GAGACATAAATATGAACACACAGGTAAAAGAC	TTGAGAATAAGACCCAGAGTGTGAGAAG
ZEB2	CCACCAGTCCAGACCAGTATTCCT	CATCAAGCAATTCTCCCTGAAATC
TWIST1	GCCGGAGACCTAGATGTCATTGT	GCCCTGTTTCTTTGAATTTGGAT
TWIST2	GCTGCCCTCTGACAAGCTGA	CTTATTGTCCATCTCGTCGCTCTG
VIM	GAAGGCGAGGAGAGCAGGATT	AGAAGTTTCGTTGATAACCTGTCCA
FOXC2	CAGCAGCAAACTTTCCCCAAC	CAGTATTTCGTGCAGTCGTAGGAGTAG
CDH-1	AGGCCAAGCAGCAGTACATT	ATTCACATCCAGCACATCCA
CD24	AAACAACAACTGGAACTTCAAGTAACTC	GGTGGTGGCATTAGTTGGATTT
CD44t	TCCAACACCTCCCAGTATGACA	GGCAGGTCTGTGACTGATGTACA
CD44v	CCACATTCTACAAGCACAA	CTGTTGCCAAACCACTGTTCC
CD44s	TCCCTGCTACCAGAGACCAAGACA	ACCAGAGGTTGTGTTTGCTCCACC
GAPDH	GAGTCAACGGATTTGGTCGTAT	ATGGGTGGAATCATATTGGAAC
TGF-beta3	AGCGGAATGAGCAGAGGATCGA	CTCAACAGCCACTCACGCACAG

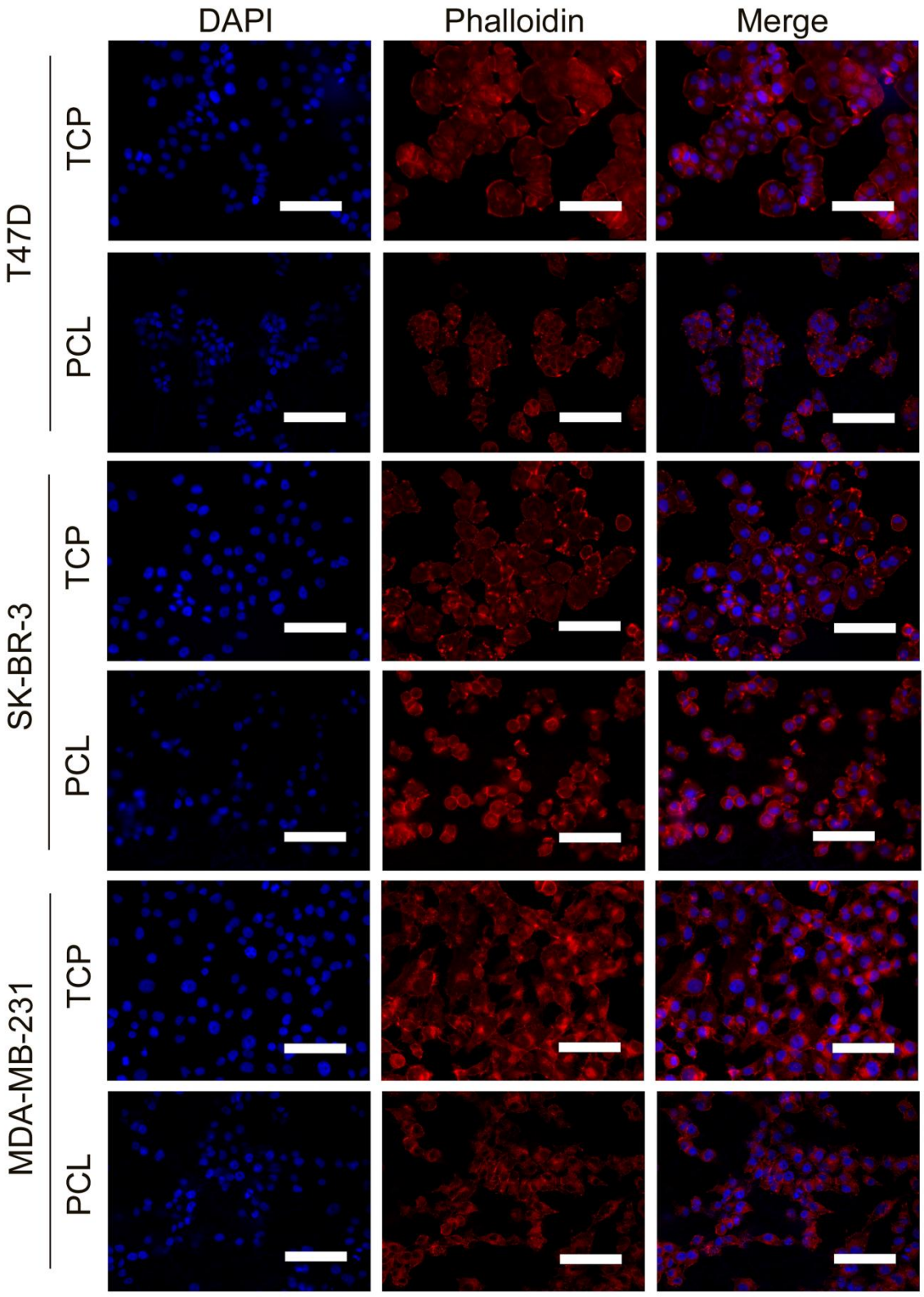

Fig. S1. Fluorescence microscopy images of T47D, SK-BR-3, and MDA-MB-231 cells cultured in 2D tissue culture plastics (TCP) and 3D PCL fibrous scaffolds (PCL). Blue indicates nuclei (DAPI); Red indicates F-actin (Rhodamine-Phalloidin). All scale bars indicate 100 μm.

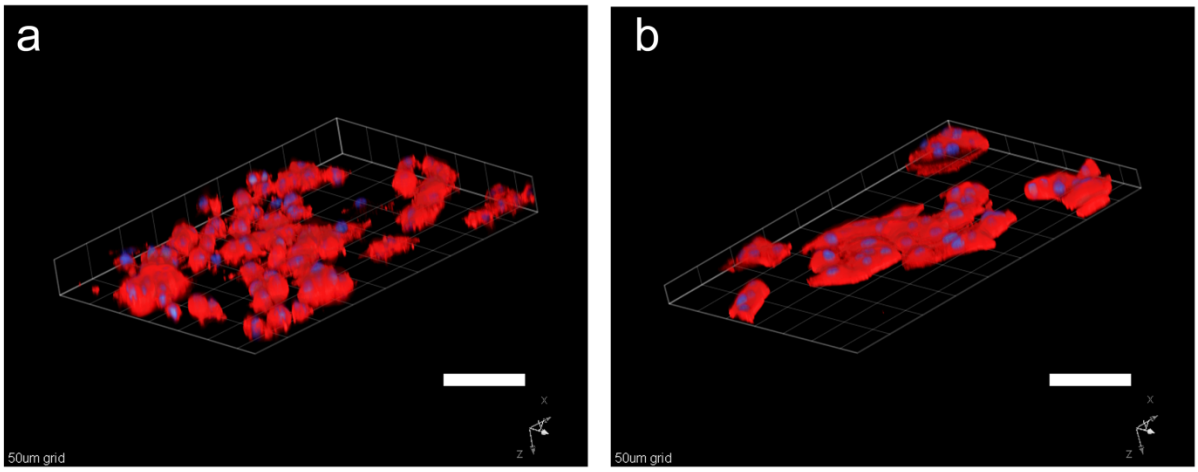

Fig. S2. MCF-7 cells seeded in PCL fibrous scaffold occupied architectural features of the matrix in three dimensions as observed using confocal microscope. 3D reconstruction of sequential strata demonstrated MCF-7 occupation of sub-surface niches within the PCL scaffold. Serial z-plane sampling was conducted for total z-scan distance of 45 μm at 3 μm per plane for cells in PCL scaffolds (a) and 20 μm at 3 μm per plane for cells on TCP (b) . Blue indicates nuclei (DAPI); Red indicates F-actin (Rhodamine-Phalloidin). All scale bars indicate 100 μm.

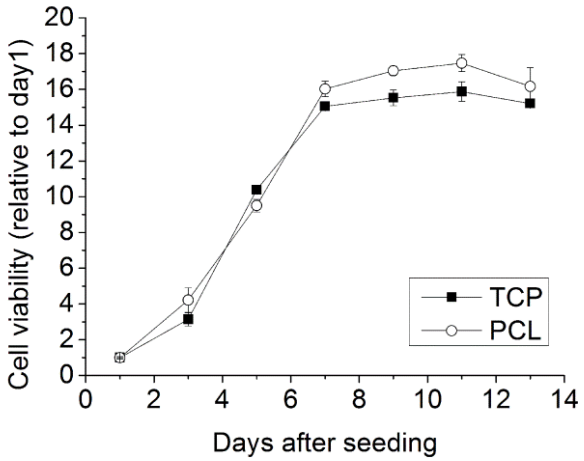

Fig. S3. The proliferations of MCF-7 in 2D tissue culture plastics (TCP) and 3D PCL fibrous scaffolds (PCL) were measured at indicated time points by CellTiter-Blue assay. Results were shown as mean ± standard deviation. Statistical significance of the differences between cells in TCP and PCL is indicated by two asterisks ($P < 0.005$, $n = 3$).

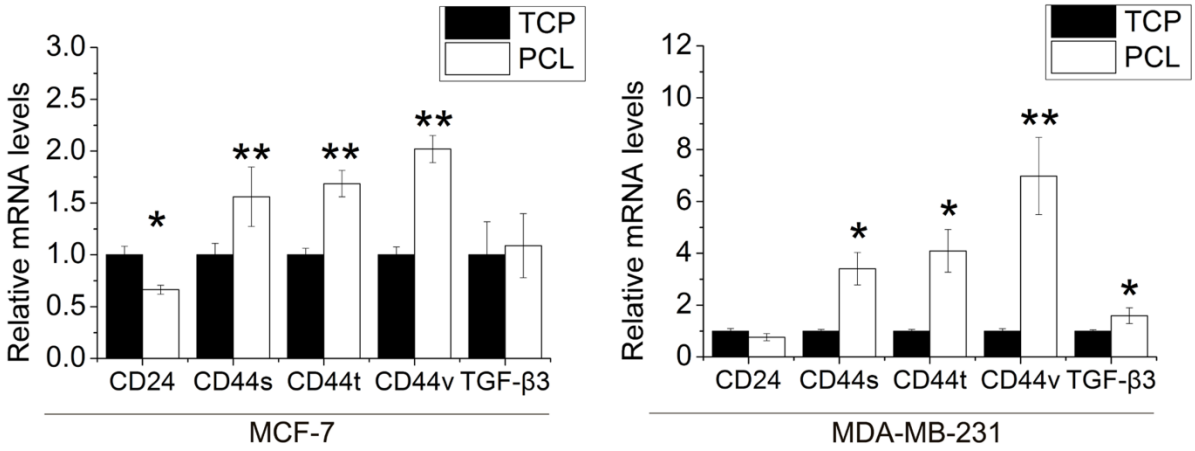

Fig. S4. qRT-PCR of CD24, CD44 variants and TGF-β3 expression in MCF-7 and MDA-MB-231 cells cultured in PCL fibrous scaffolds (PCL) compared to cells on TCP. Results were shown as mean± standard deviation. Statistical significance is indicated by single asterisk ($P < 0.05$, $n = 3$) or double asterisks ($P < 0.005$, $n = 3$).

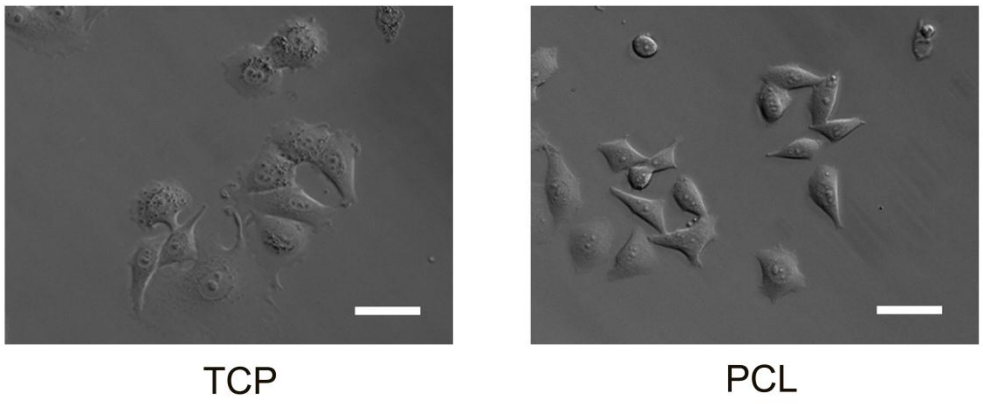

Fig. S5. Cells from PCL fibrous scaffolds (PCL) culture displayed a mesenchymal morphology after being re-plated in TCP (right), relative cells from TCP (left). Scale bars indicate 50 μm.

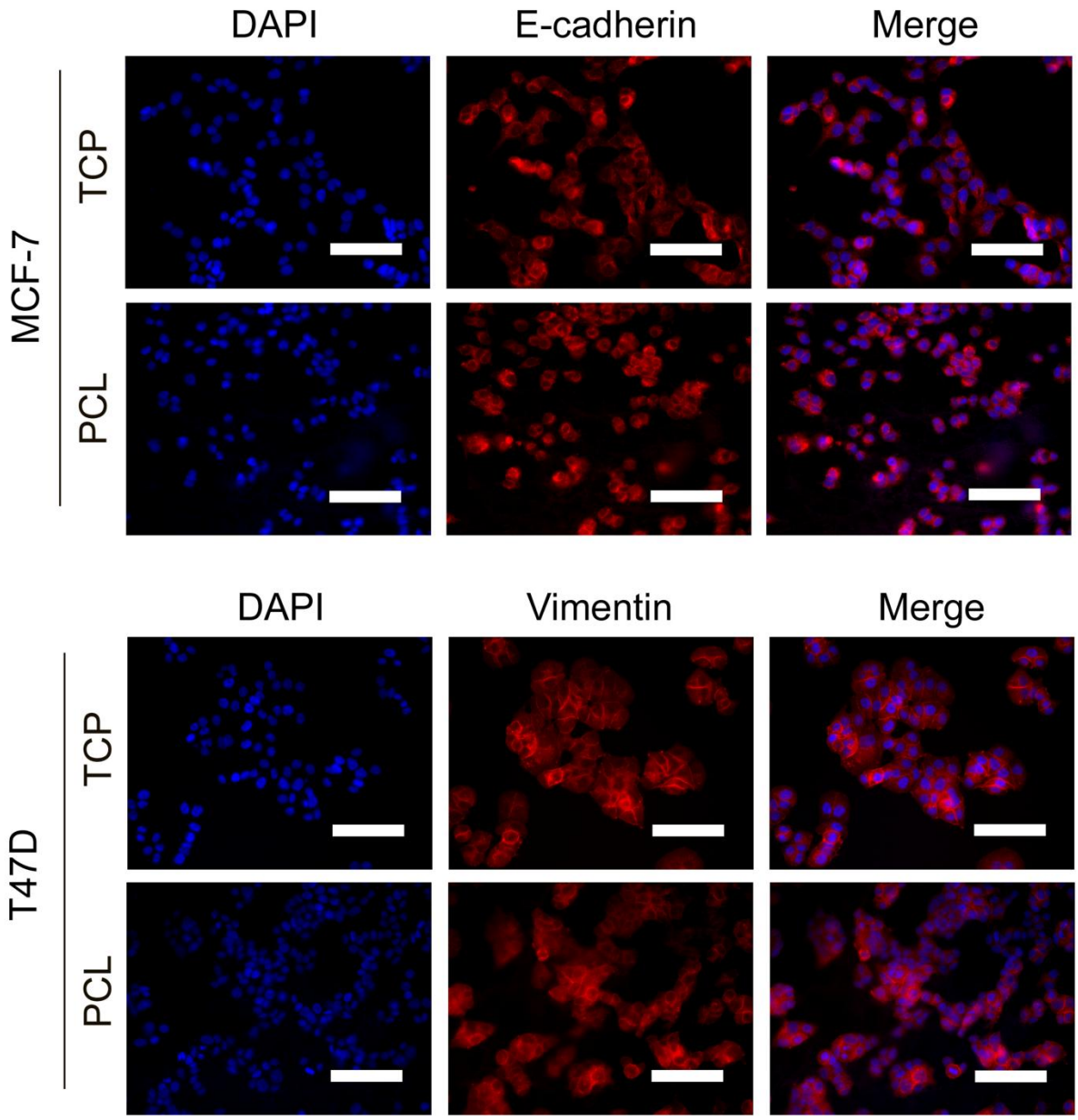

Fig. S6. Immunofluorescence staining for *E*-cadherin in MCF-7 and T47D cells cultured in TCP and PCL fibrous scaffolds (PCL). Blue indicates nuclei (DAPI); red indicates *E*-cadherin. All scale bars indicate 100 μm.

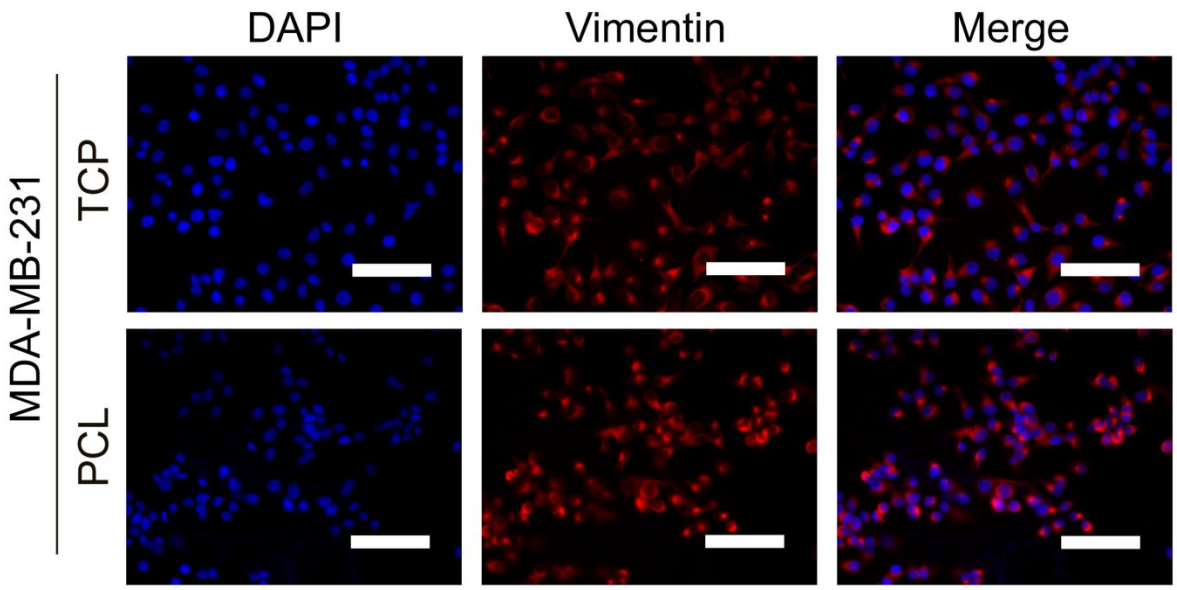

Fig. S7. Immunofluorescence staining for vimentin in MDA-MB-231 cells cultured in 2D tissue culture plastics (TCP) and 3D PCL fibrous scaffolds (PCL). Blue indicates nuclei (DAPI); Red indicates vimentin. All scale bars indicate 100 μm.

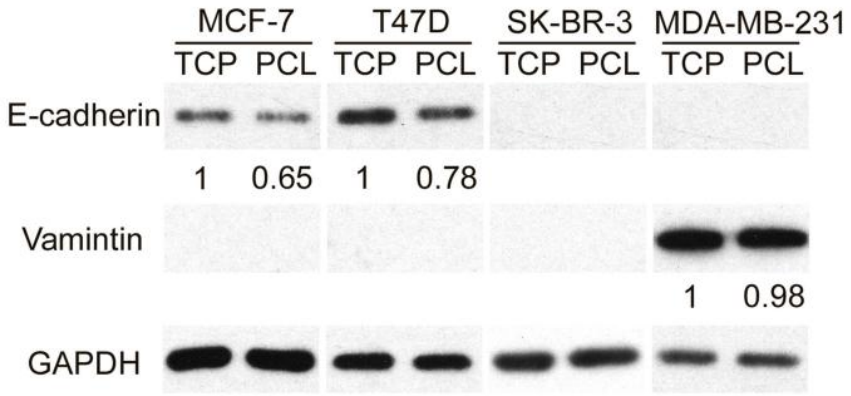

Fig. S8. After culturing for 6 days on PCL fibrous scaffolds (PCL) and TCP, cell extracts were used for Western blot analysis to detect the expression of *E*-cadherin and Vimentin. The density of each band was quantified with The Image J software and divided by the density of the corresponding GAPDH band. The ratio is presented under each blot after normalizing the values for TCP control as one unit.

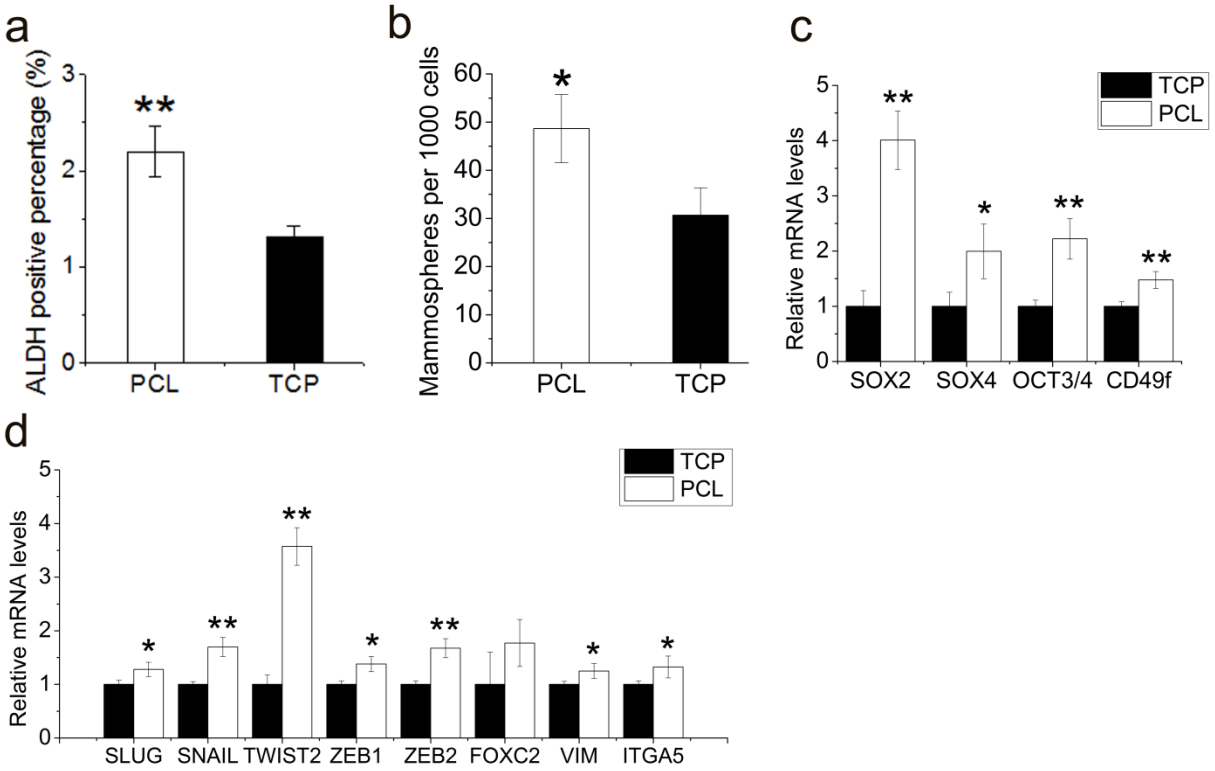

Fig. S9. MDA-MB-231 cells cultured in PCL fibrous scaffolds displayed increased CSCs properties and gene expression pattern of EMT. (a) The percentage of ALDH-positive cells in MDA-MB-231 cells cultured in TCP and PCL Results were shown as mean ± standard deviation. Statistical significance is indicated by double asterisks ($P < 0.001$, $n = 3$). (b) MDA-MB-231 cultured in PCL and TCP were trypsinized and then plated in non-adherent conditions in mammosphere medium, as described in Methods. Mammospheres were numerated. Results were shown as mean ± standard deviation. Statistical significance is denoted by single asterisks ($P < 0.05$, $n = 3$). (c) qRT-PCR analysis was used to quantify the expression of stem cell markers, SOX2, SOX4, OCT3/4, and CD49f. (d) qRT-PCR analysis was used to quantify the expression of EMT markers in MDA-MB-231 from PCL and TCP. Results were shown as mean± standard deviation. Statistical significance is indicated by single asterisk ($P < 0.05$, $n = 3$) or double asterisks ($P < 0.005$, $n = 3$).

Supplementary Movie Legend

Movie S1: MCF-7 cells were cultured on PCL fibrous scaffolds for 4 days. Cells were stained with DAPI (nucleus) and Rhodamine-Phalloidin (F-actin). The series movie was generated by serially displaying sequential strata taken on a confocal microscope in 3 μm intervals along the z-axis. The movie shows that some of cells migrated into the under layer of fibrous scaffolds. Scale bar indicates 100 μm.

References for Supplemental Material

1. D. Guo, K. Hu, Y. Lei, Y. Wang, T. Ma, D. He, *J. Biol. Chem.*, 2004, **279**, 53498-53505.