Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

PLGA-collagen-ECMs hybrid scaffolds functionalized with biomimetic extracellular matrices secreted by mesenchymal stem cells during stepwise osteogenesis-co-adipogenesis

Yazhou Chen [a,b], Kyubae Lee [a,b], Naoki Kawazoe [a], Yingnan Yang [c] and Guoping Chen [a,b,*]

[a] Research Center of Functional Materials, National Institute for Materials Science,1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan

[b] Department of Materials Science and Engineering, Graduate School of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8571, Japan

[c] Graduate School of Life and Environmental Science, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8571, Japan.

*Corresponding author:
Email: Guoping.Chen@nims.go.jp; Fax: +81-29-860-4673; Tel: +81-29-860-4496

Supporting Figures

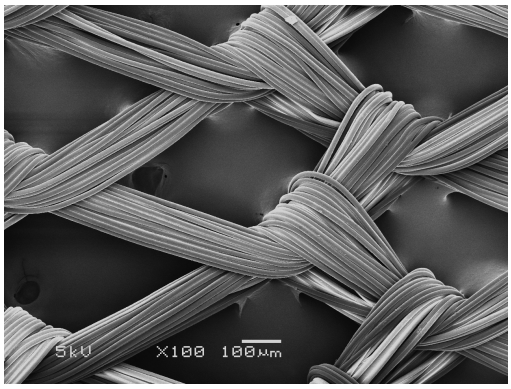

Figure S1. SEM image of PLGA mesh.

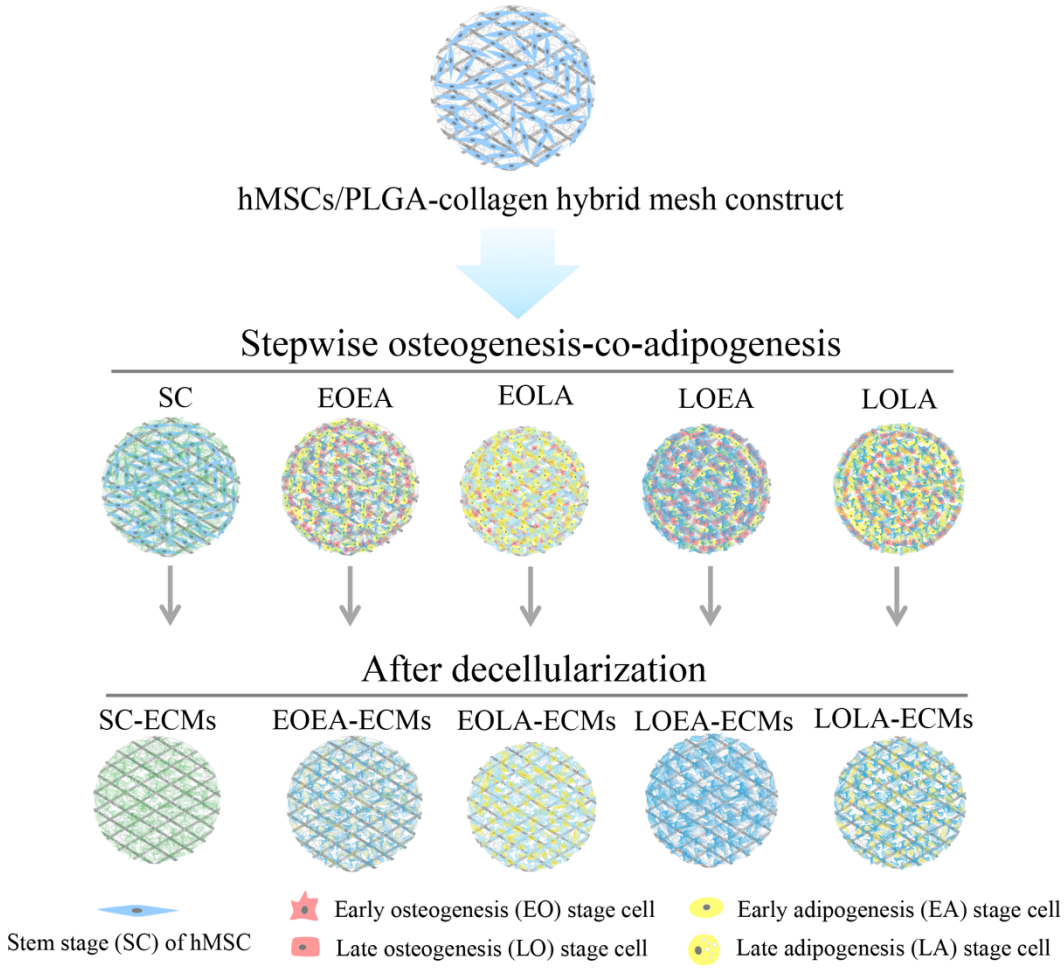

Figure S2. Preparation scheme of PLGA-collagen-ECMs hybrid meshes by decellularizing hMSCs/PLGA-collagen hybrid mesh constructs. hMSCs were controlled at different stages of osteogenesis-co-adipogenesis.

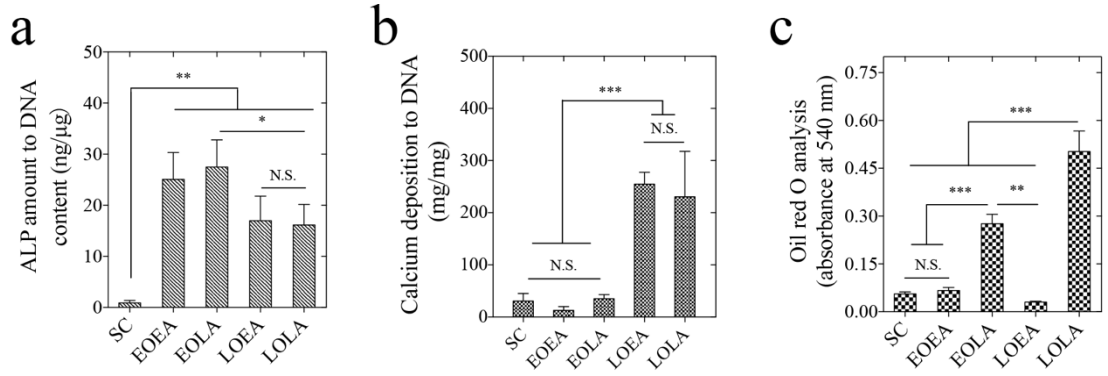

Figure S3. Quantitative analysis of ALP activity (a), calcium deposition (b) and Oil red O staining (c). Date represent means ± S.D. (n = 3). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. N.S., no significant difference.

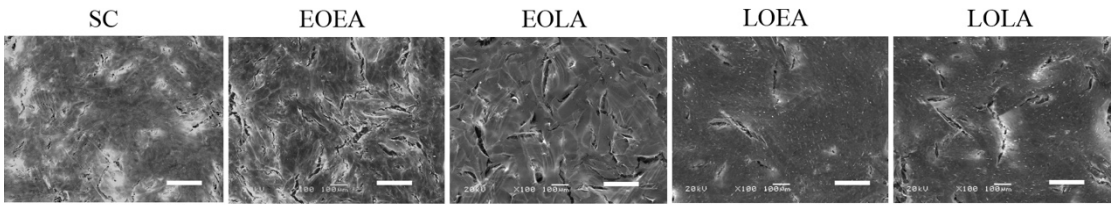

Figure S4. SEM images of the hMSCs/PLGA-collagen hybrid mesh constructs after stepwise osteogenic-co-adipogenic differentiation. Scale bar: 200 μm.

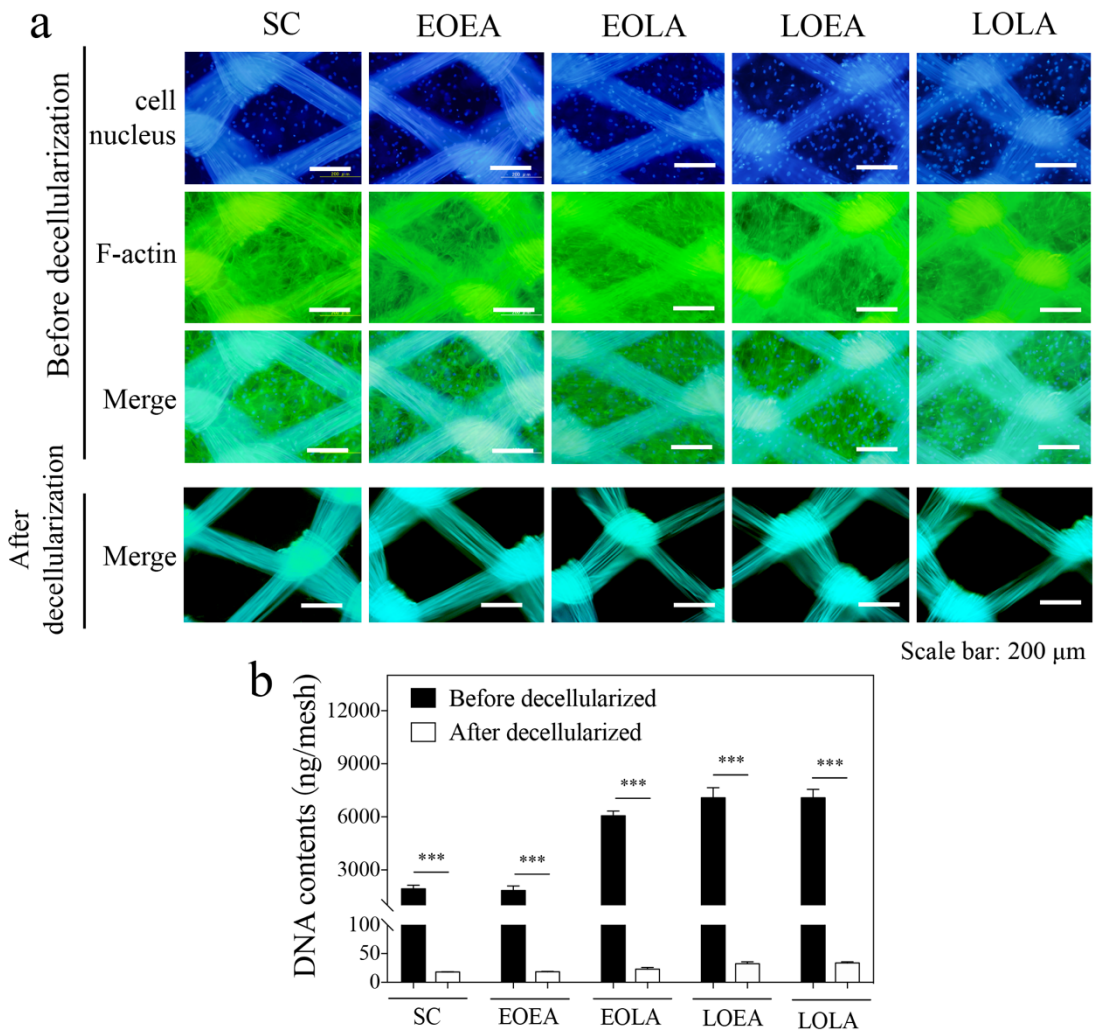

Figure S5. Decellularization of the hMSCs/PLGA-collagen hybrid mesh constructs. Cell nucleus and f-actin staining of the hMSCs/PLGA-collagen constructs before and after decellularization (a). DNA quantification of the hMSCs/PLGA-collagen hybrid mesh constructs before and after decellularization (b).

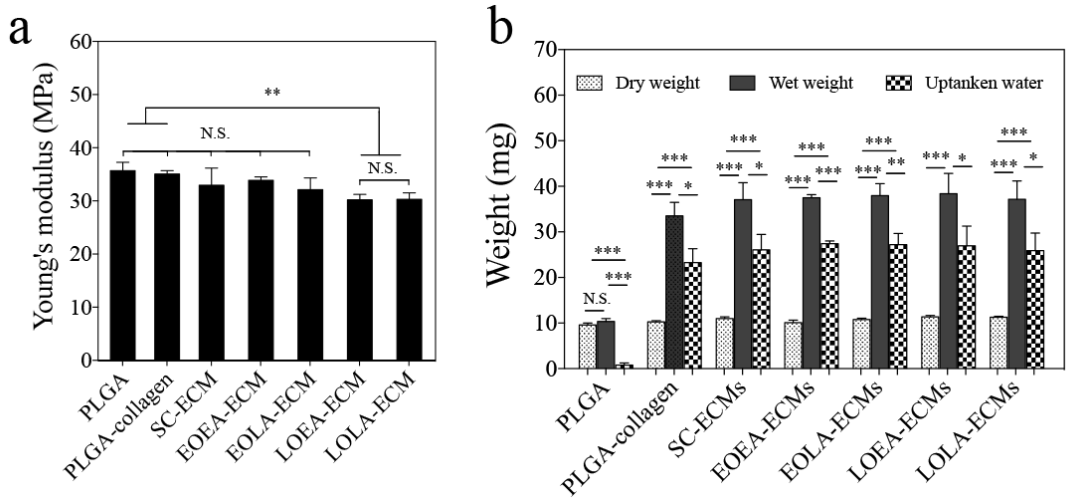

Figure S6. The mechanical properties and water uptake of PLGA-collagen and ECM-scaffolds groups.

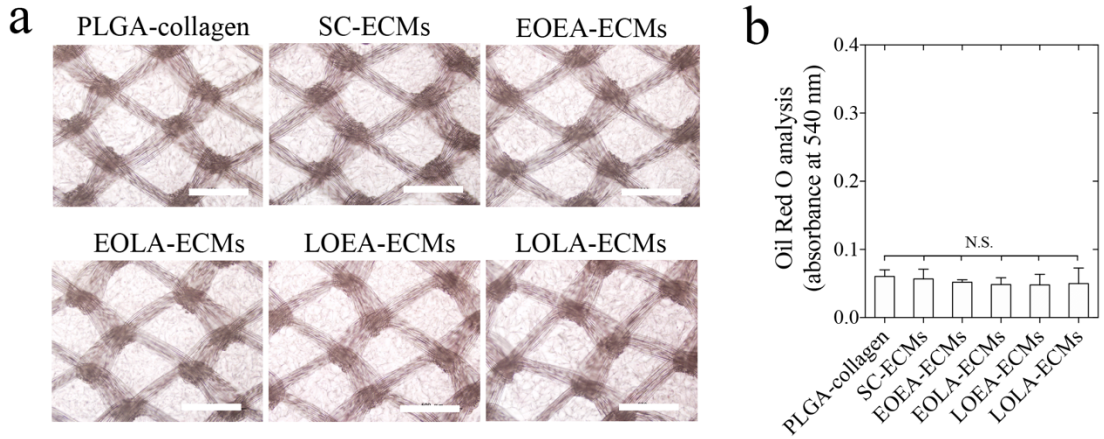

Figure S7. Measurement of the lipid vacuoles in the cells/ECMs-scaffold and cells/PLGA-collagen constructs when being cultured in basal medium. Qualitative analysis of the constructs by Oil red O staining after culture in basal medium (a), and quantitative analysis of the lipid vacuoles dye after culture in basal medium (b). Date represent means ± S.D. (n = 3). *, $P < 0.05$; N.S., no significant difference. Scale bar: 500 μm.

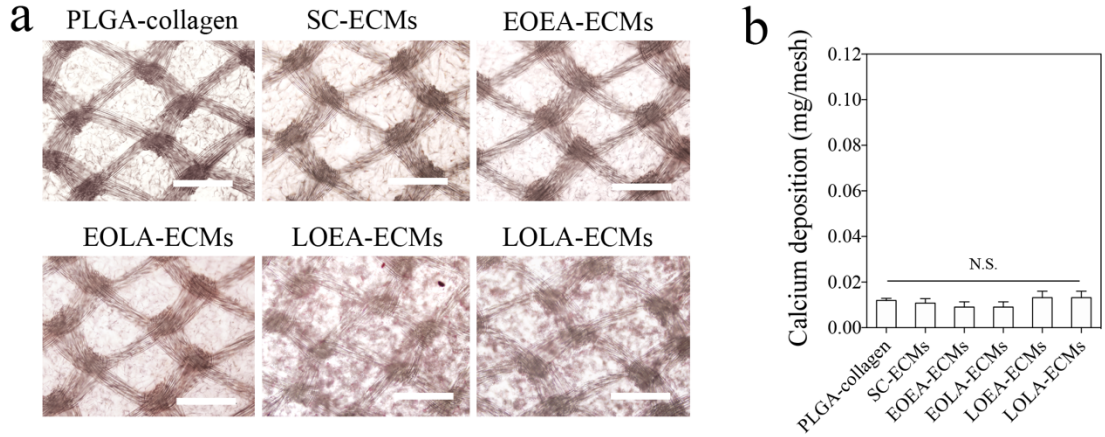

Figure S8. Measurement of the calcium deposition in the cells/ECMs-scaffold and cells/PLGA-collagen constructs when being cultured in basal medium. Qualitative analysis of the constructs by alizarin red S after culture in basal medium (a), and quantitative analysis of the calcium deposits after culture in basal medium (b). Date represent means ± S.D. (n = 3). *, $P < 0.05$; N.S., no significant difference. Scale bar: 500 μm.

Supporting Tables

Table S1. Decellularization efficiency based on DNA quantification.

SC	EOEA	EOLA	LOEA	LOLA
99.1 ± 0.1%	98.9 ± 0.3%	99.6 ± 0.8%	99.4 ± 0.1%	99.3 ± 0.4%

Table S2. Primers and probes for real-time PCR analysis.

mRNA	Description	Oligonucleotide
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	Hs99999905_m1
		Forward 5'-GACCCTTGACCCCCACAAT-3'
ALP	Alkaline phosphatase	Reverse 5'-GCTCGTACTGCATGTCCCCT-3'
		Probe 5'-TGGACTACCTATTGGGTCTCTTCGAGCCA-3'
		Forward 5'-TGCCTTGAGCCTGCTTCC-3'
IBSP	Bone sialoprotein 2	Reverse 5'-GCAAAATTAAAGCAGTCTTCATTTTG-3'
		Probe 5'-CTCCAGGACTGCCAGAGGAAGCAATCA-3'
		Forward 5'-CTCAGGCCAGTTGCAGCC-3'
SPP1	Secreted phosphoprotein 1	Reverse 5'-CAAAAGCAAATCACTGCAATTCTC -3'
		Probe 5'-AAACGCCGACCAAGGAAAACTCACTACC-3'
SP7	Osterix	Hs00541729_m1
RUNX2	Runt-related transcription factor-2	Hs00231692_m1
PPARG	Peroxisome proliferator-activated receptor gamma	Hs01115510_m1
LPL	Lipoprotein lipase	Hs00173425_m1
FABP4	Fatty acid binding protein 4	Hs00609791_m1
FASN	Fatty acid synthase	Hs00188012_m1
CEBPA	CCAAT/enhancer binding protein	Hs00269972_s1