Supporting Information

Enhanced osteo-angiogenic coupling by a bioactive cell-free fat extract (CEFFE) delivered through electrospun fibers

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qRT-PCR

Table S1 shows the forward and reverse primers used for the gene expression analysis, as described in **Section 2.3.6** of the main text.

Table S1. Primer sequences of osteogenesis-related genes for qRT-PCR

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
GAPDH	CAAGCTCATTTCCTGGTATGACAAT	GTTGGGATAGGGCCTCTCTTG
Col1a1	TTCCTGAAGATGTCGTTGATGTG	TGTTTTTGCAGTGGTATGTGTAATGTTC
Runx-2	GACTGTGGTTACCGTCATGGC	ACTTGGTTTTTCATAACAGCGGA
OPN	AGAGCGGTGAGTCTAAGGAGT	TGCCCTTTCCGTTGTTGTCC
OCN	GAACAGACAAGTCCCACACAGC	TCAGCAGAGTGAGCAGAAAGAT

MTT assay

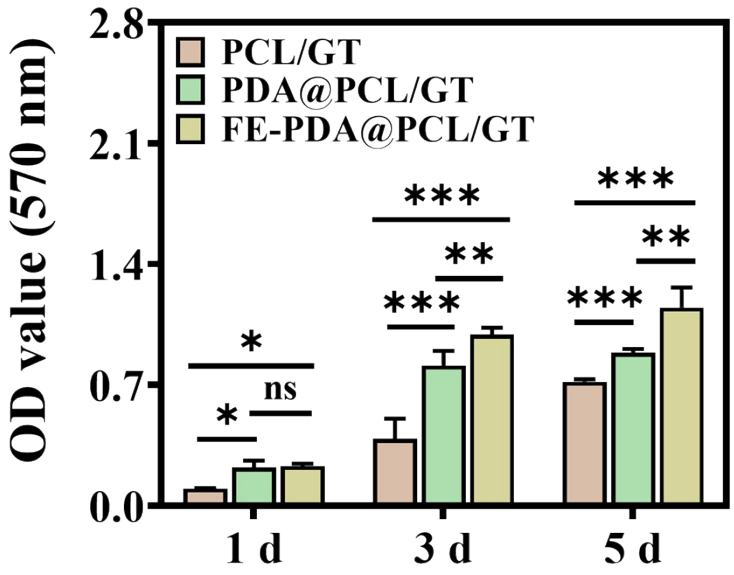


Figure S1. Proliferation of MC3T3-E1 cells cultured on the fibrous scaffolds of PCL/GT, PDA@PCL/GT, and FE-PDA@PCL/GT for 1, 3, 5 days, n=4.

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Beyotime, China) was performed to assess cell proliferation. Briefly, MC3T3-E1 cells at a density of 1.0×10^4 cells per well were seeded on the fibrous scaffolds of PCL/GT,

PDA@PCL/GT, and FE-PDA@PCL/GT in 24-well culture plates. After culturing for 1, 3, and 5 days, 500 μL of working medium, prepared by diluting MTT in medium at a volume ratio of 1:10, was added to each well and incubated at 37 °C for 4 h. Next, 500 μL of formazan lysis solution was introduced to each well followed by continuous incubation until the formazan was completely dissolved. Lastly, 100 μL volume of the solution was transferred to a 96 well plate reader (MK3, Thermo, USA) for absorbance measurements at 570 nm. **Figure S1** shows that while all the fibrous scaffolds supported the MC3T3-E1 cells' proliferation, cells cultured on the FE-PDA@PCL/GT exhibited the most pronounced proliferative ability.

2D collective cell migration assay

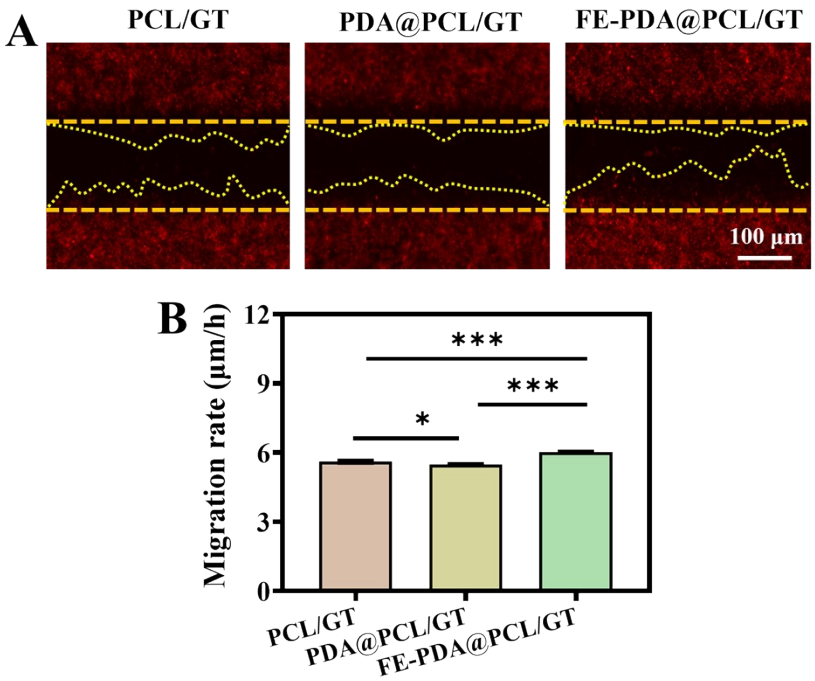
An "artificial wound" method [1] was used to examine the collective cell migration of MC3T3-E1 cells. Briefly, stainless-steel rings (ϕ = 15 mm) with 1 mm thickness of barrier bar in the middle were placed on the fibrous scaffolds of PCL/GT, PDA@PCL/GT, and FE-PDA@PCL/GT in 24-well plates. MC3T3-E1 cells, prior stained with CellTracker Red CMFDA (MKBio, China), were then seeded at seeding density of 5.0×10^5 cells per well. After 3 days of culturing, the rings were removed to initiate cell invading to the cell-free area for 24 h. Thereafter, the cells were fixed with 4% paraformaldehyde for observation and the cell migration rate was determined by:

$$\text{Cell migration rate } (\mu\text{m h}^{-1}) = \frac{S_0 - S_t}{2Lt} \qquad (1)$$

where S_0 and S_t are the areas of cell-free region before and after cell migration, respectively; L is the width of the analyzed images and t is the period of time for cell migration (24 h).

It was found that cells migrated into the denuded area was most effective for the FE-PDA@PCL/GT group (**Figure S2A**). Quantified migration rate of cells on the fibrous substrates were in the order of FE-PDA@PCL/GT (6.03 ± 0.04 μm h^{-1}) > PCL/GT

(5.61 ± 0.07 μm h^{-1}) > PDA@PCL/GT (5.48 ± 0.04 μm h^{-1}), with statistically significant differences between the FE-PDA@PCL/GT group and the other two groups



(**Figure S2B**).

Figure S2. (A) Collective cell migration by "wound healing" assay, with the two parallel orange dashed lines showing the initial cell-free zones and the two wave-like yellow dotted lines showing the frontiers of cell migration; (B) Quantified cell migration rate in (A), n=5.

mRNA expression of angiocrine factors in huvECs

huvECs (5.0 × 10^4 cells per well) were seeded on the fibrous scaffolds of PCL/GT, PDA@PCL/GT, and FE-PDA@PCL/GT in 24-well plates (**Figure S3A**). After 3 days of culture, qRT-PCR was performed to detect mRNA expression of angiocrine factors including bone morphogenetic protein-2 (*BMP-2*), bone morphogenetic protein-7 (*BMP-7*), Wnt family member 1 (*Wnt1*), and Wnt family member 5a (*Wnt5a*) in huvECs. Total RNA isolation, cDNA synthesis, and qRT-PCR were conducted in accordance with the protocol detailed in **Section 2.3.6** of the main text. Gene expression level was normalized to the endogenous housekeeping GAPDH. The forward and

reverse primers for the selected genes are listed in **Table S2**.

The results showed that the expression of osteogenesis-related genes was significantly upregulated for the cells cultured on the FE-PDA@PCL/GT, with the levels of *BMP-2*, *BMP-7*, and *Wnt1* genes being 1.5-, 1.3-, and 1.2-fold higher than that in the PCL/GT group, respectively (**Figure S3B**). This finding is consistent with previous studies, showing that ECs secreted some angiocrine factors with osteogenic property, such as Notch, WNT, and BMP [2-4].

Table S2. Primer sequences of osteogenesis-related genes

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
GAPDH	GGACCTGACCTGCCGTCTAG	GTAGCCCAGGATGCCCTTGA
BMP-2	ACCCGCTGTCTTCTAGCGT	TTTCAGGCCGAACATGCTGAG
BMP-7	TCGGCACCCATGTTCATGC	GAGGAAATGGCTATCTTGCAGG
Wnt1	CGATGGTGGGGTATTGTGAAC	CCGGATTTTGGCGTATCAGAC
Wnt5a	ATTCTTGGTGGTCGCTAGGTA	CGCCTTCTCCGATGTACTGC

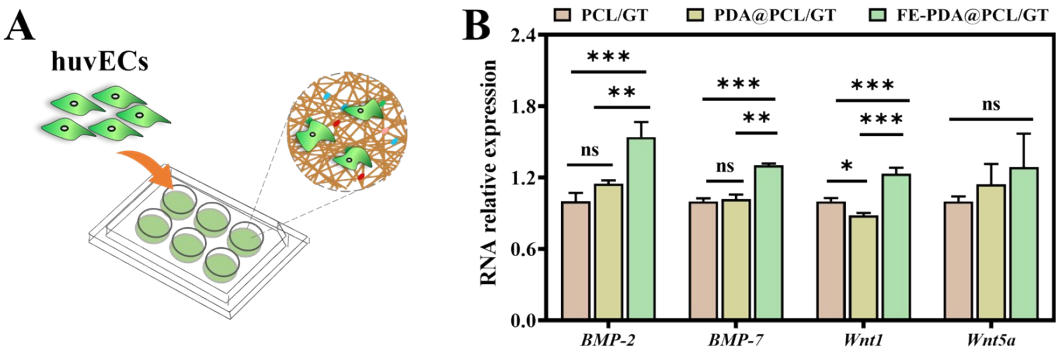


Figure S3. Fibrous FE-PDA@PCL/GT scaffold promoted the mRNA expression of osteogenic factors in huvECs: (A) huvECs were seeded onto fibrous scaffolds of PCL/GT, PDA@PCL/GT and FE-PDA@PCL/GT for 3 days of culturing; (B) Gene expression of *BMP-2*, *BMP-7*, *Wnt1* and *Wnt5a*, n=3.

mRNA expression of angiogenic factors

MC3T3-E1 cells (5.0×10^4 cells per well) were seeded on the fibrous scaffolds of PCL/GT, PDA@PCL/GT, and FE-PDA@PCL/GT in 24-well plates (**Figure S4A**). After 3 days of culture, qRT-PCR was performed to detect mRNA expression of

angiogenic factors including stromal cell-derived factor-1α (*SDF-1α*), C-X-C chemokine receptor type 4 (*CXCR4*), vascular endothelial growth factor (*VEGF*) and basic fibroblast growth factor (*bFGF*) in MC3T3-E1 cells. Total RNA isolation, cDNA synthesis, and qRT-PCR were conducted in accordance with the protocol detailed in **Section 2.3.6** of the main text. Gene expression level was normalized to the endogenous housekeeping GAPDH. The forward and reverse primers for the selected genes are listed in **Table S3**.

It was found that gene expression for the cells cultured on FE-PDA@PCL/GT was the highest compared to the counterpart groups. In particular, the expression levels of bFGF and VEGF in the FE-PDA@PCL/GT group were increased by 165% and 296%, respectively, compared with the PCL/GT group. Presence of FE thus significantly promoted the expression of angiogenic factors in osteoblasts. This is consistent with the previous reports showing that pro-angiogenic factors secreted by osteoblasts affected angiogenesis [5-7].

Table S3. Primer sequences of angiogenesis-related genes

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
GAPDH	CAAGCTCATTTCCTGGTATGACAAT	GTTGGGATAGGGCCTCTCTTG
bFGF	GCGACCCACACGTCAAACTA	CCGTCCATCTTCCTTCATAGC
CXCR4	GACTGGCATAGTCGGCAATG	AGAAGGGGAGTGTGATGACAAA
SDF-1α	TGCATCAGTGACGGTAAACCA	CACAGTTTGGAGTGTTGAGGAT
VEGF	GCACATAGAGAGAATGAGCTTCC	CTCCGCTCTGAACAAGGCT

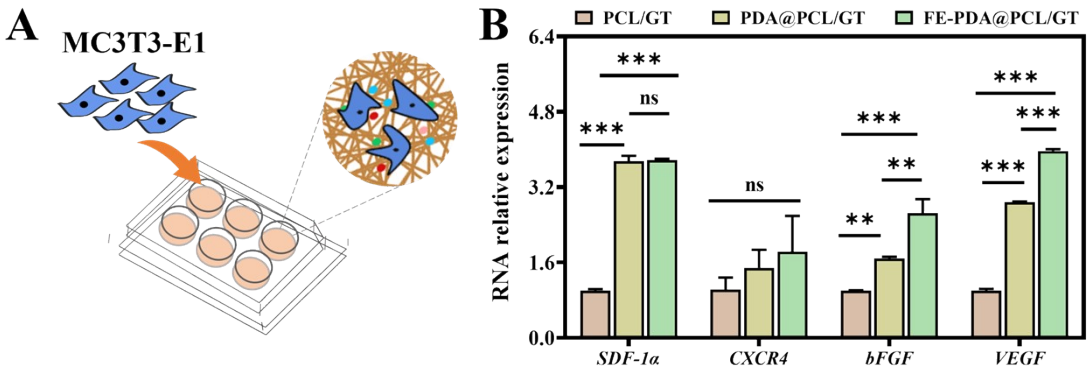


Figure S4. Fibrous FE-PDA@PCL/GT scaffold promoted mRNA expression of pro-angiogenic factors in MC3T3-E1 cells: (A) Cells were seeded onto fibrous scaffolds of

PCL/GT, PDA@PCL/GT and FE-PDA@PCL/GT for 3 days of culturing; (B) Gene expression of *SDF-1α*, *CXCR4*, *bFGF* and *VEGF*, n=3.

References

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