Supporting Information

3D Printed Polycaprolactone/Phosphoester-Modified Poly (amino acids)-Graphene Oxide Scaffold for Meniscal Regeneration

Zeping Yu, [abc] *Fei Xing,* [d] *Jianshu Li,* [efg] *Wenli Zhang,* [ab] *Hong Duan,* [*ab] *Jian Li*[*ab], *and Yan Xiong*[*ab]

[a] Sports Medicine Center, West China Hospital, Sichuan University, Chengdu 610041, China

[b] Department of Orthopedics and Orthopedic Research Institute, West China Hospital, Sichuan University, Chengdu 610041, China

[c] Department of Pediatric Orthopedics, West China Second University Hospital, Sichuan University, Chengdu, 610041, China

[d] Department of Pediatric Surgery, Laboratory of Stem Cell and Tissue Engineering, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu 610041, China.

[e] Med-X Center for Materials, Sichuan University, Chengdu 610041, China

[f] College of Polymer Science and Engineering, State Key Laboratory of Polymer Materials Engineering, Sichuan University, Chengdu 610065, China

[g] State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu 610041, P. R. China

[*]Corresponding author

Email Address: **luyibingli@163.com (Yan Xiong)**; hxlijian.china@163.com (Jian Li); and duanhong1970@126.com (Hong Duan).

Materials: Antibodies used in this study were summarized as follows. Primary antibody COL I (67288-1-lg, Proteintech, USA), COL II (NB600-844, Novus Biologicals, USA), Alexa Fluor® 647-conjugated CD206 (MCA2235A647, BIO-RAD, USA), iNOS(22226-1-AP, Proteintech, USA), CD31(11265-1-AP, Proteintech, USA). Secondary antibody CoraLite488-conjugated Goat Anti-Rabbit IgG (H+L) (SA00013-1, Proteintech, USA), CoraLite594 – conjugated Goat Anti-Rabbit IgG (H+L) (SA00013-4, Proteintech, USA). YSFluor™ 488 Donkey Anti-Rat lgG (H+L) (33106ES60, YEASEN, China) was used for Immunofluorescence double staining of RAW264.7. HRP-conjugated Goat Anti-Mouse IgG (H+L) (SA00001-1, Proteintech, USA) was used for immunohistochemical staining. CD206 Monoclonal Antibody (Clone # MR6F3, PE-conjugated, eBioscience, USA). CD86 Monoclonal Antibody (Clone # GL1; APC-conjugated, eBioscience, USA).

Experimental Section

Effect of PCL/P-P-GO scaffold on macrophage-mediated angiogenesis in vitro

All experiments in this section were conducted with three duplicates for each group.

Conditioned medium (CM) preparation: CM was obtained by co-culture of different scaffolds with macrophages for 48 h.

Wound scratch assay: HUVECs (8×10^5 per well) were seeded in 6-well plates. Once confluent, the monolayer was wounded with a 200 μL pipette tip, aided by a straightedge, and the wells were rinsed three times with PBS before incubation with CM for 24 hours. Wound closure was documented at 0 and 24 hours using a Leica DMi8 inverted fluorescence microscope, and the healing ratio was determined using ImageJ software.

Transwell assay: To determine the migratory potential of HUVECs induced by the scaffolds, a Transwell assay was conducted with PCL, PCL/PE-m-PAAs, and PCL/P-P-GO. A 200 μL suspension containing 2×10^4 HUVECs in serum-free DMEM was

placed in the upper compartment of a 24-well Transwell plate (8 μm pore size, Corning, USA), with the lower compartment receiving 600 μL of complete medium and the respective scaffolds. The control group had 600 μL of complete medium in the lower chamber without scaffolds. After 4-h incubation, migrated cells were fixed using 4 % paraformaldehyde (Biosharp, China) for 15 min followed by staining with crystal violet solution (Solarbio, China) for 5 min and calculated using Image J.

Cell tube formation assessment: For the tube formation assay, 96-well plates were pre-coated with 50 μL of Matrigel (Corning, USA), followed by the seeding of HUVECs at 2×10^4 cells/well. Cultured for 6 hours under different conditions, the assay evaluated tube formation by quantifying nodes, branch counts, and total tube lengths.

Immunofluorescence Staining: HUVECs were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 in PBS for 30 minutes. They were then blocked with 5% BSA (Sigma, USA) for 1 hour before being incubated with a CD31 antibody overnight at 4°C. After three PBS washes, cells were incubated with CoraLite488-conjugated goat anti-rabbit secondary antibody for 1 hour. Following further PBS rinses, DAPI staining was applied. Images were captured using a fluorescence microscope (Nikon, Japan), and semi-quantitative MFI analysis was conducted with ImageJ.

Supplementary results:

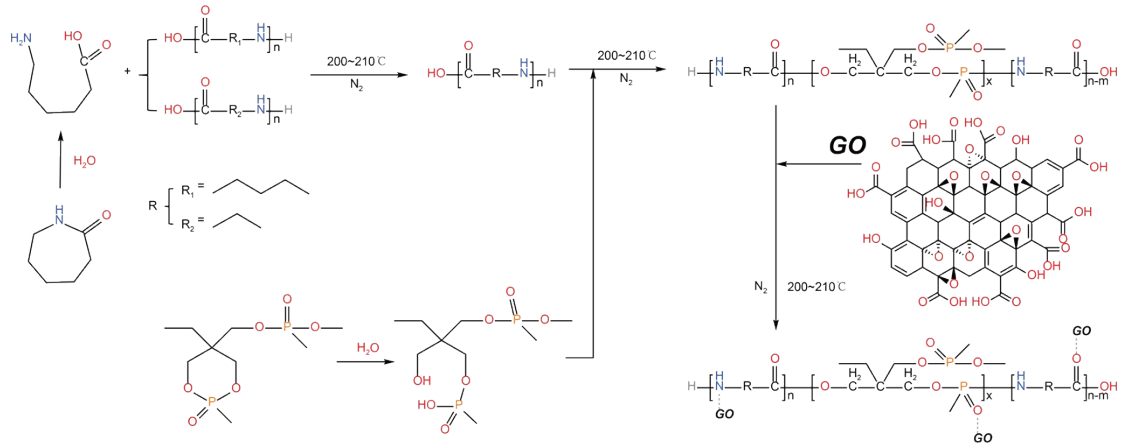

Fig. S1 The synthetic route of PE-m-PAAs and P-P-GO.

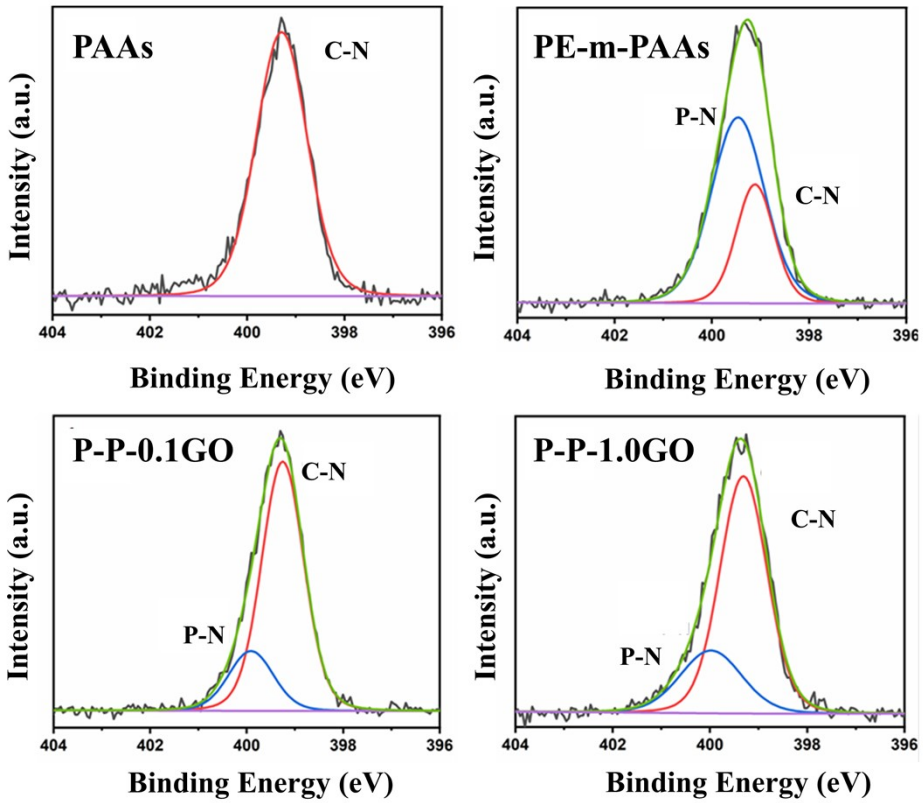

Fig. S2 N 1s XPS spectrum of PAAs, PE-m-PAAs, and P-P-GO containing 0.1, 1.0 wt%, respectively.

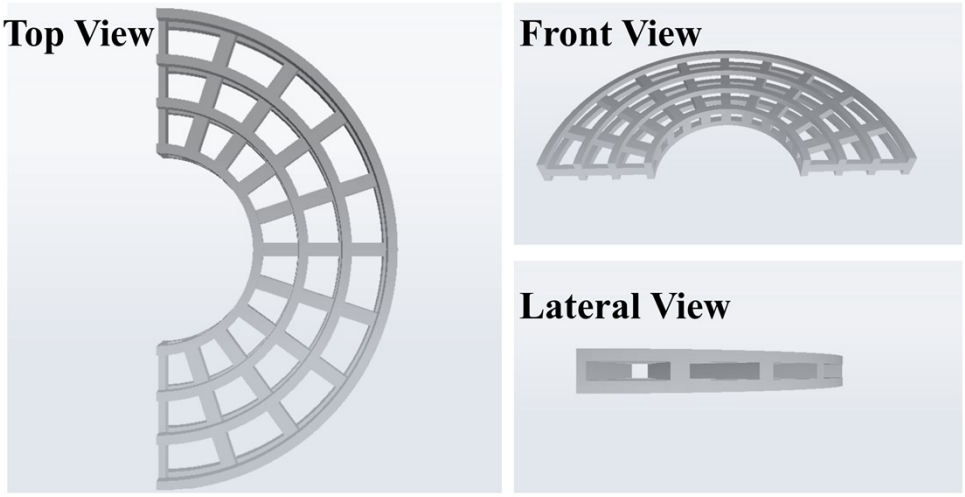

Fig. S3 The designed 3D model with circumferentially and radially oriented fibers, which simulated the natural collagen fiber arrangement within the native meniscus.

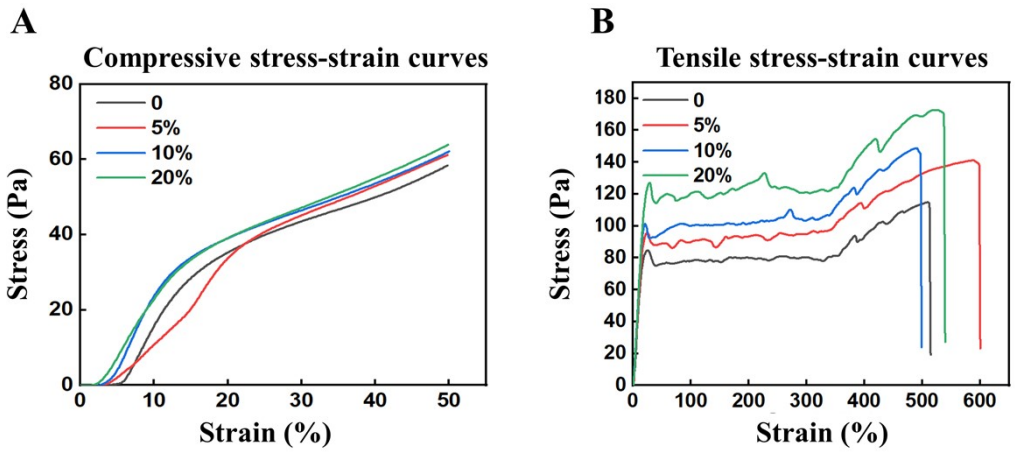

Fig. S4 Compressive (A) and tensile (B) stress–strain curves of the PCL containing various wt% of PE-m-PAAs.

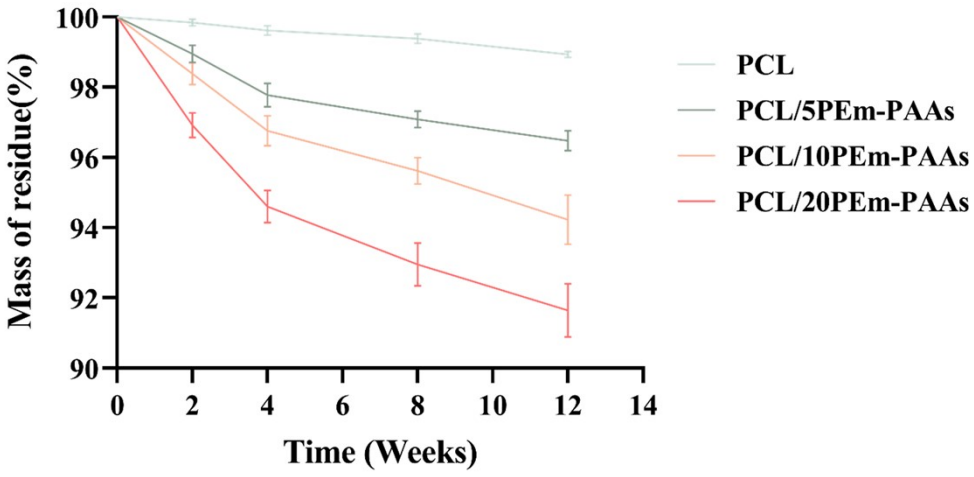

Fig. S5 Degradation rate of the PCL and PCL/PE-m-PAAs scaffolds containing various wt% of PE-m-PAAs (n = 5).

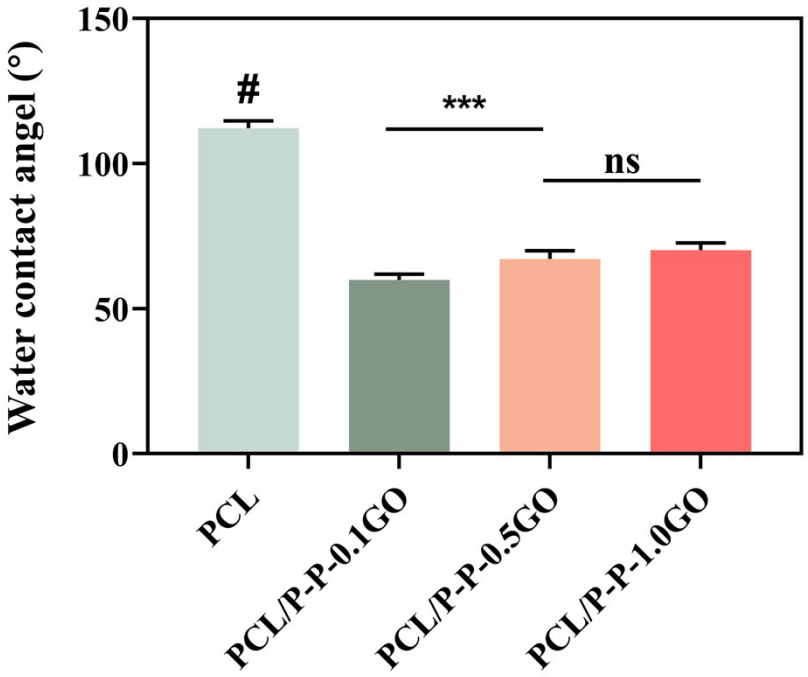

Fig. S6 Water contact angle of PCL and PCL/P-P-GO containing various wt% of GO (n = 5; ns, no significance; $^{\#}$, $p < 0.05$ compared with any other group; ***, $p < 0.001$).

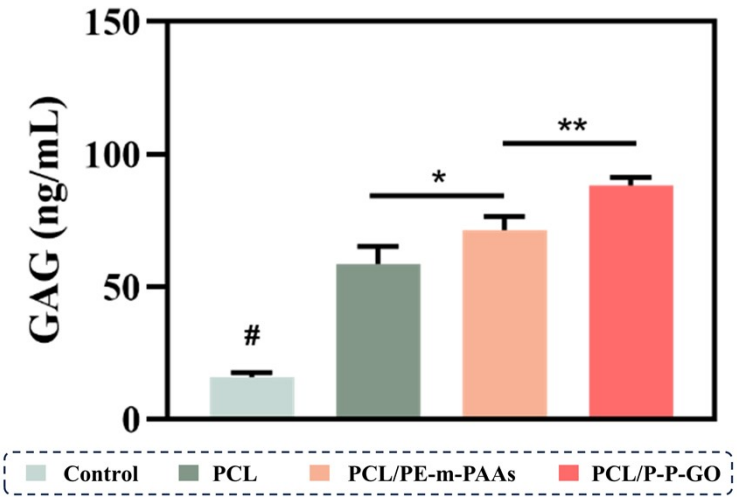

Fig. S7 ELISA analysis of GAG of BMCs on various scaffolds (n = 3; $^{\#}$, $p < 0.05$ compared with any other group; *, $p < 0.05$; **, $p < 0.01$).

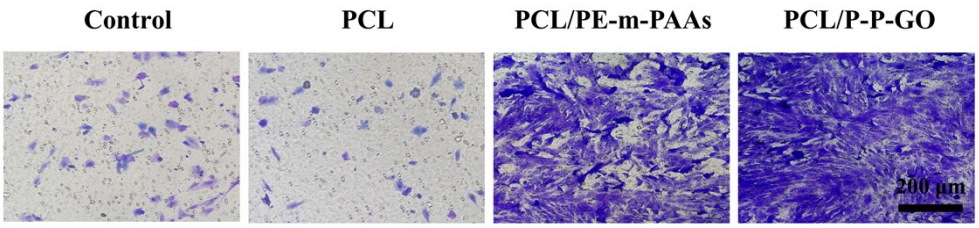

Fig. S8 Transwell migration assay of SMSCs treated with various scaffolds for 24 h.

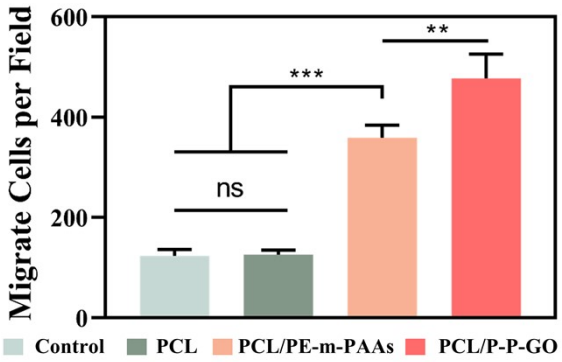

Fig. S9 Transwell analysis of migrated SMSCs of scaffolds (n = 3; ns, no significance; **, $p < 0.01$; ***, $p < 0.001$).

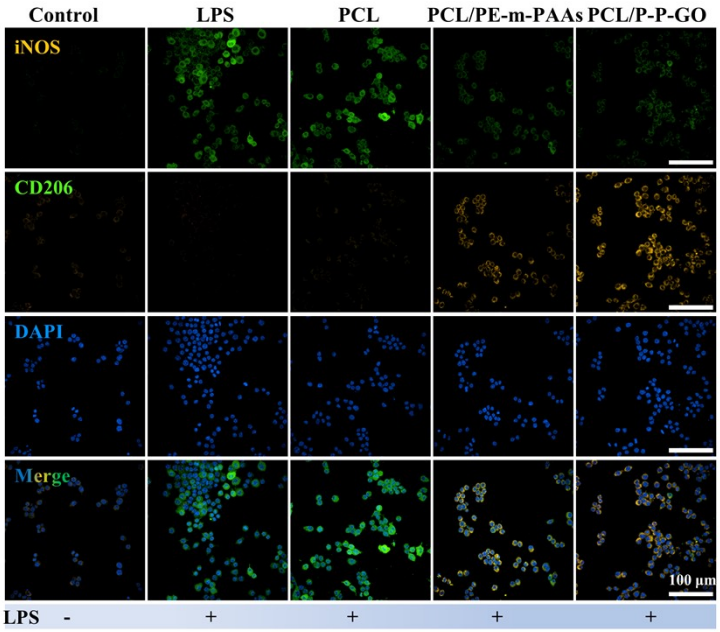

Fig. S10 Immunofluorescence staining a of M1 (iNOS) and M2 (CD206) in LPS-stimulated RAW264.7 cells co-incubating with different scaffolds.

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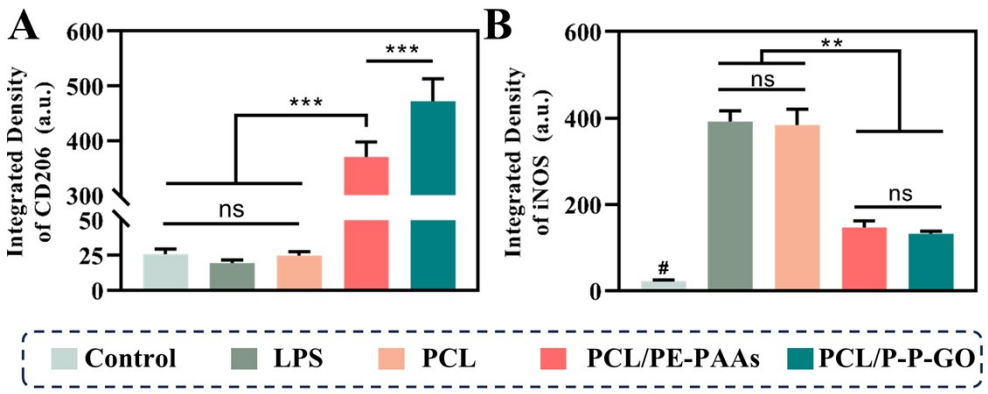

Fig. S11 MFI of (A) M2 (CD206) and (B) M1 (iNOS) in LPS-stimulated RAW264.7 cells co-incubating with different scaffolds (n = 3; ns, no significance; $^{\#}$, $p < 0.05$ compared with any other group; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

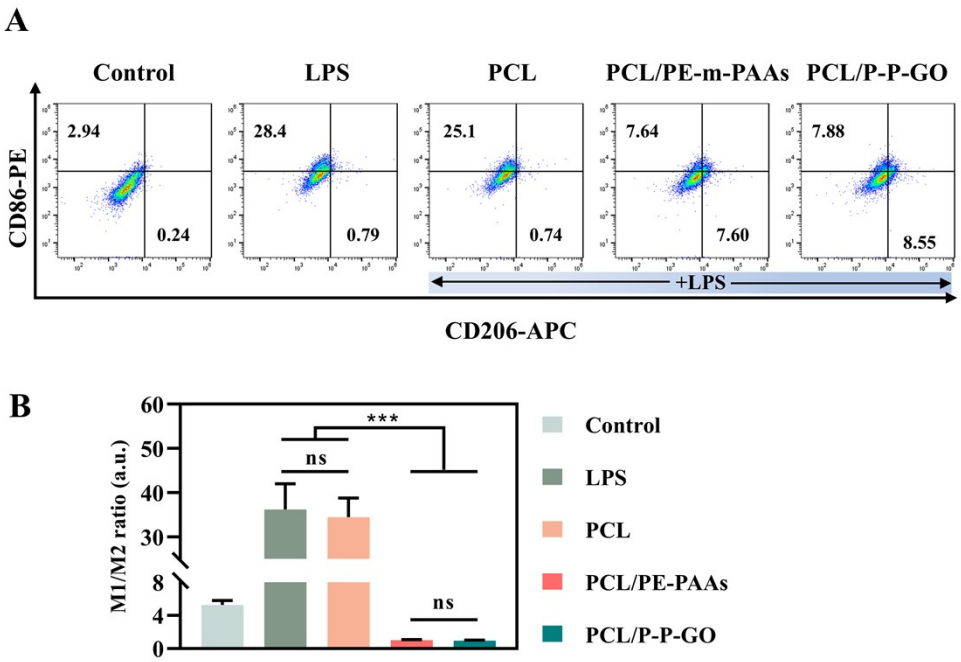

Fig. S12 (A) Flow cytometry results of macrophages expressing CD86 and CD206 after treatment with different scaffolds. The antibodies of CD86 (PE channel) and CD206 (APC channel) were employed to specifically label M1 macrophages and M2 macrophages, respectively. (B) Quantitative analysis of M/M2 ratio (n = 3; ns, no significance; ***, $p < 0.001$).

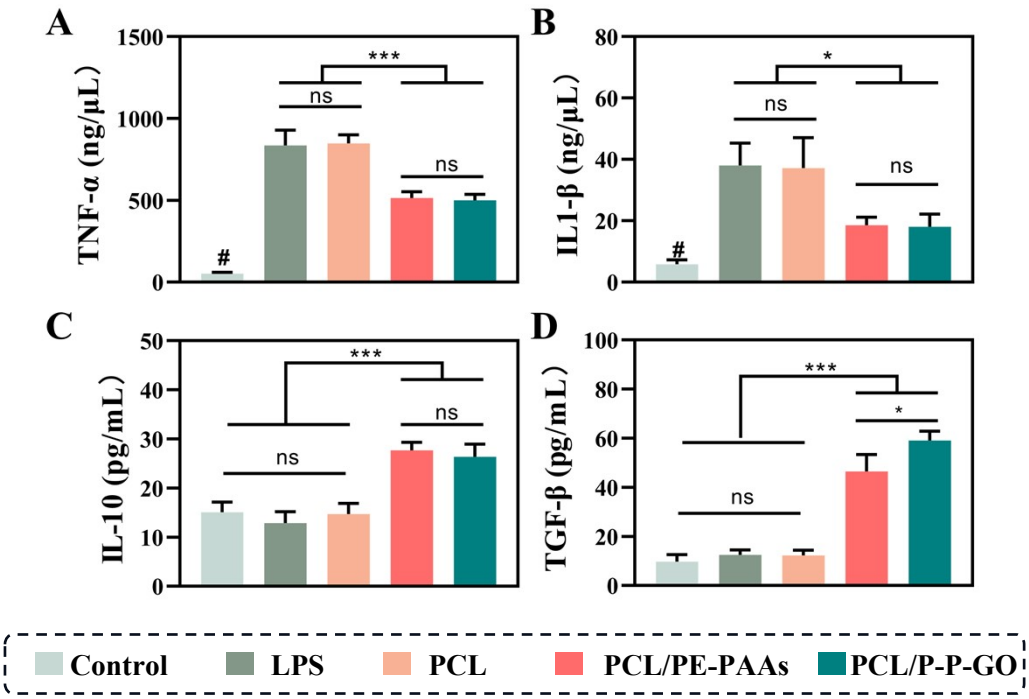

Fig. S13 ELISA analysis of (A) TNF-α, (B) IL1-β, (C) IL-10, (D) TGF-β in LPS-stimulated RAW264.7 cells co-incubating with different scaffolds (n = 3; $^{\#}$, $p < 0.05$ compared with any other group; *, $p < 0.05$; ***, $p < 0.001$).

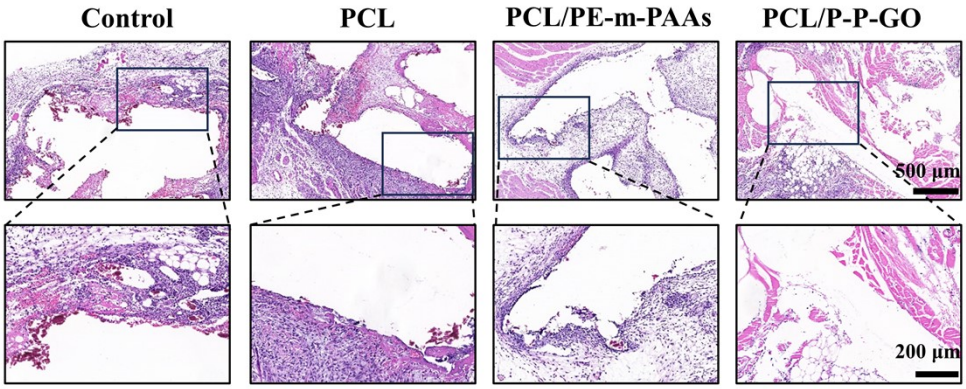

Fig. S14 H&E staining images of the different scaffolds implanted in the subcutaneous muscular tissue of rabbits.

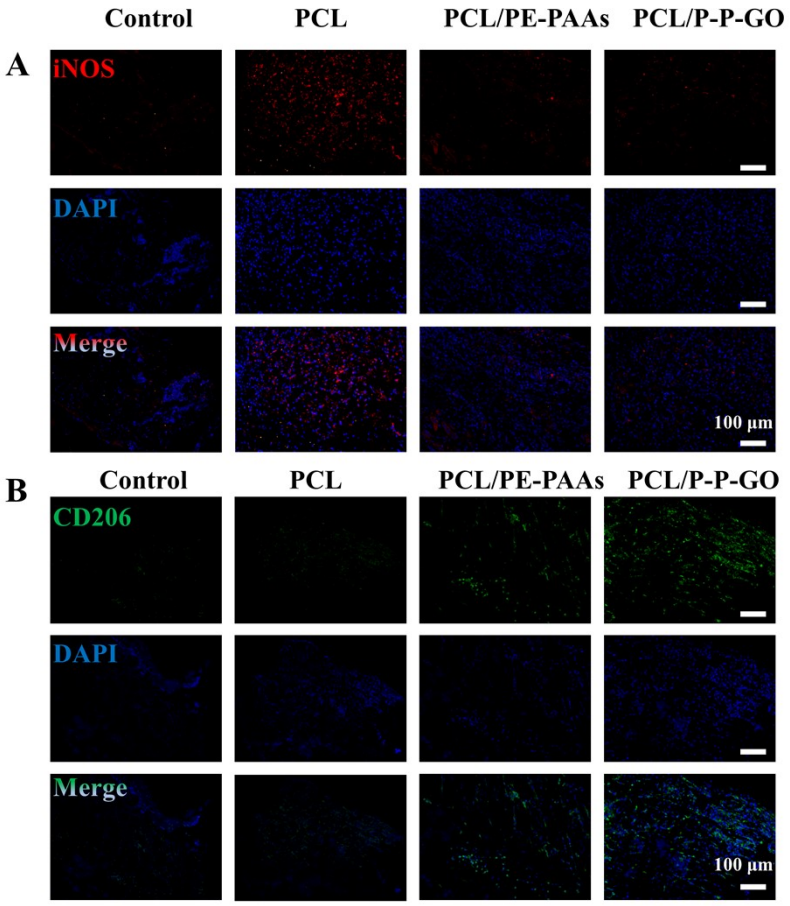

Fig. S15 Representative immunofluorescence images of (A) M1 (iNOS) and (B) M2 (CD206) macrophages in the knee synovium of rats one-month post-implantation of various scaffolds.

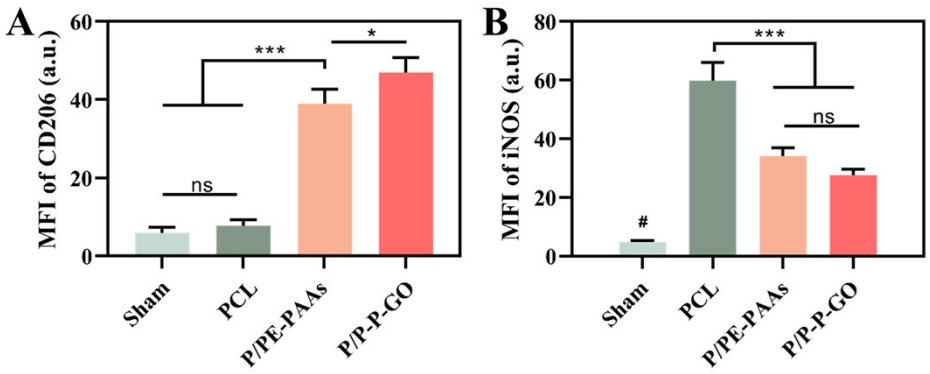

Fig. S16 MFI of (A) M2 (CD206) and (B) M1 (iNOS) macrophages in the knee synovium of rats one-month post-implantation of various scaffolds (n = 3; ns, no significance; #, $p < 0.05$ compared with any other group; *, $p < 0.05$; ***, $p < 0.001$).

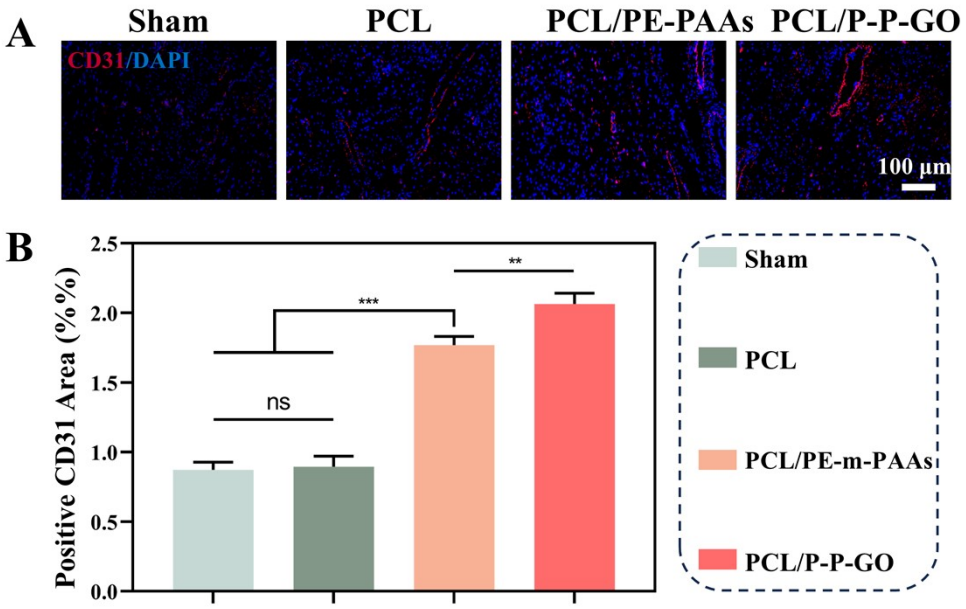

Fig. S17 (A) Representative immunofluorescence images and (B) MFI of CD31 in the knee synovium of rabbits after 6 months post-implantation of various scaffolds (n = 3; ns, no significance; **, $p < 0.01$; ***, $p < 0.001$).

Table S1 The primer sets used in RT–PCR

Gene name		Primers (5' to 3')
COL I	Forward	CCTGCTGGTCCTGCTGGTC
COL I	Reverse	TATGCCTCTGTCGCCCTGTTC
COL II	Forward	CACGCTCAAGTCCCTCAACAAC
COL II	Reverse	TCTATCCAGTAGTCACCGCTCTTC
SOX 9	Forward	GCTCCAGCCTCTATTCCACC
SOX 9	Reverse	TGGTGAGCTGTGTGTACACC
Aggrecan	Forward	TGGAGAAGCCCTTGCATCTG
Aggrecan	Reverse	AGCATAGGCAGATGTCTCGC
GAPDH	Forward	CCATCACCATCTTCCAGGAG
GAPDH	Reverse	GATGATGACCCTTTTGGCTC