Supplementary Information

Peptide-Based Inflammation-Responsive Implant Coating Sequentially Regulates Bone Regeneration to Enhance Interfacial Osseointegration

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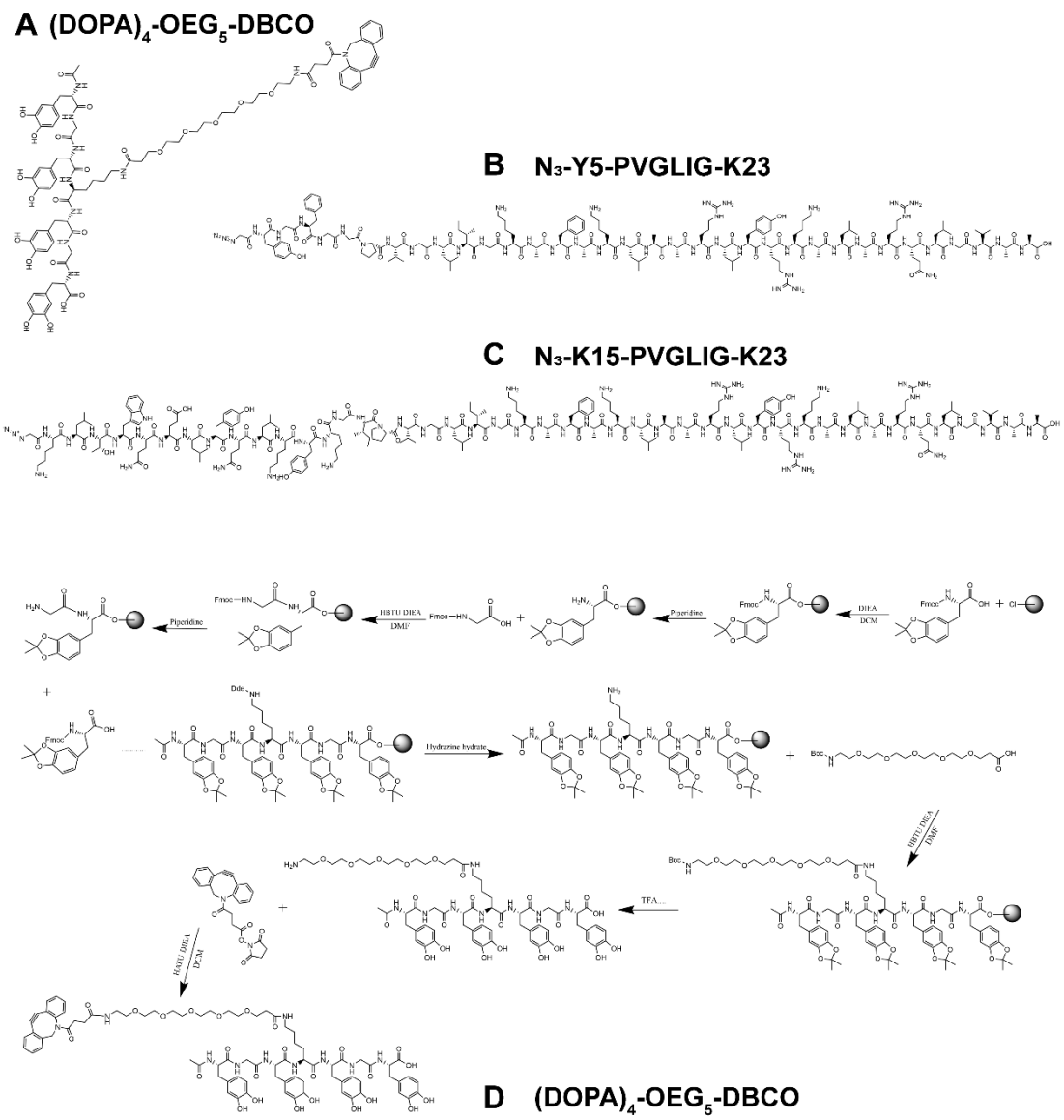
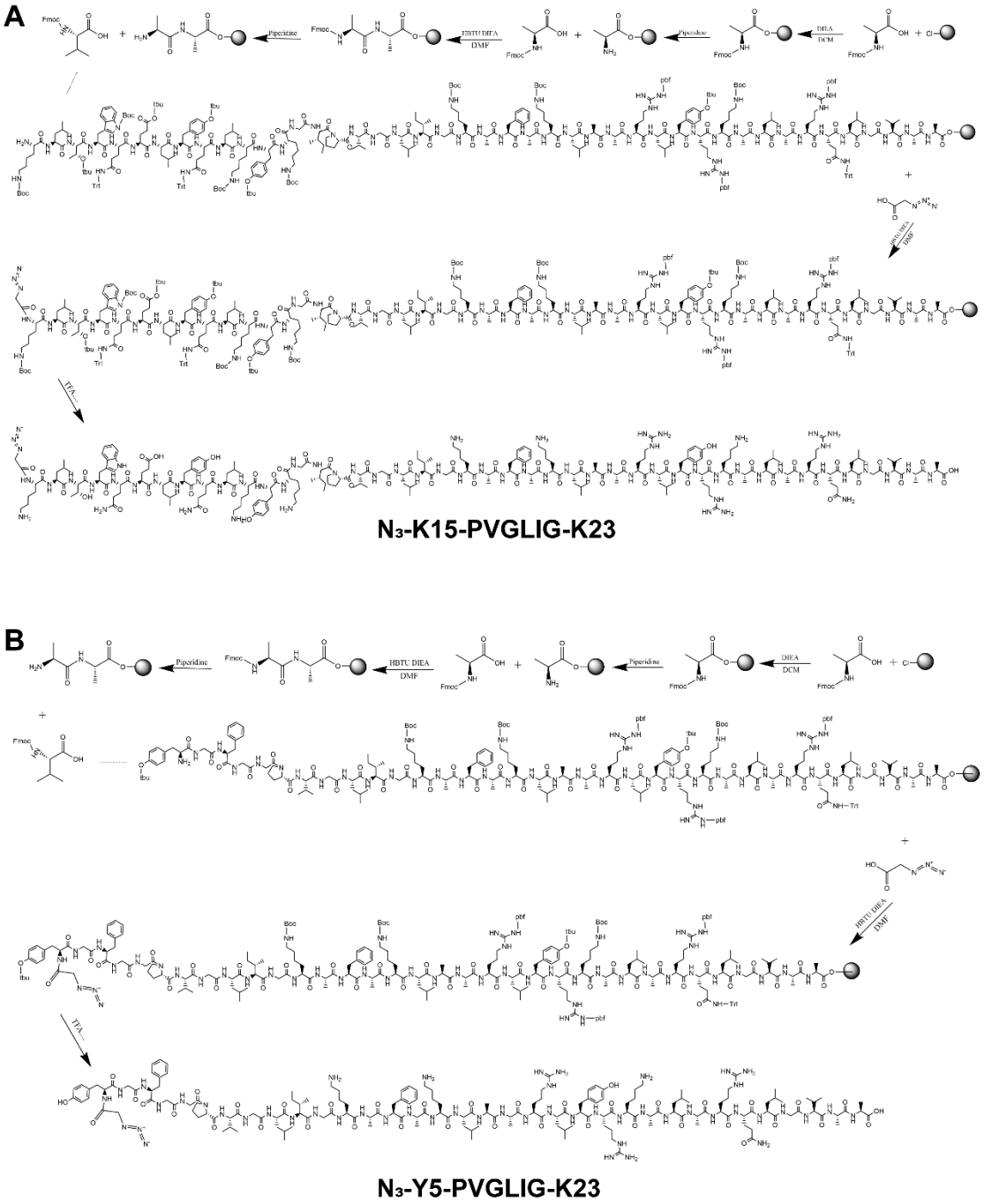
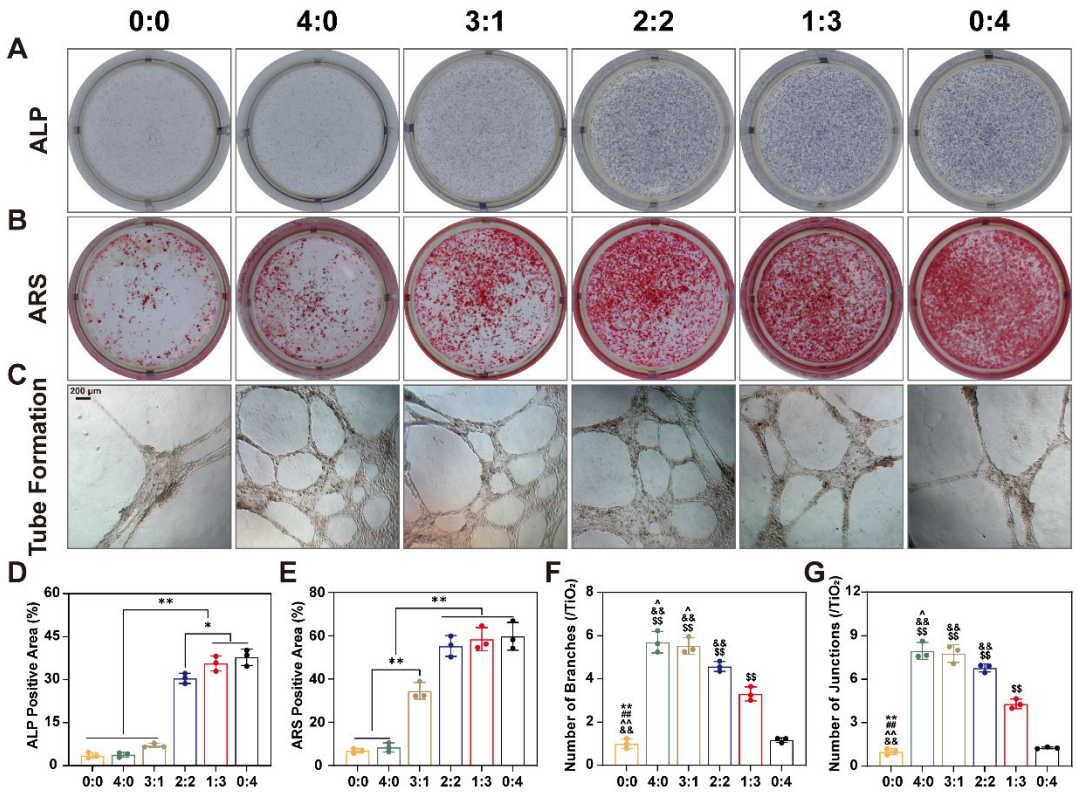


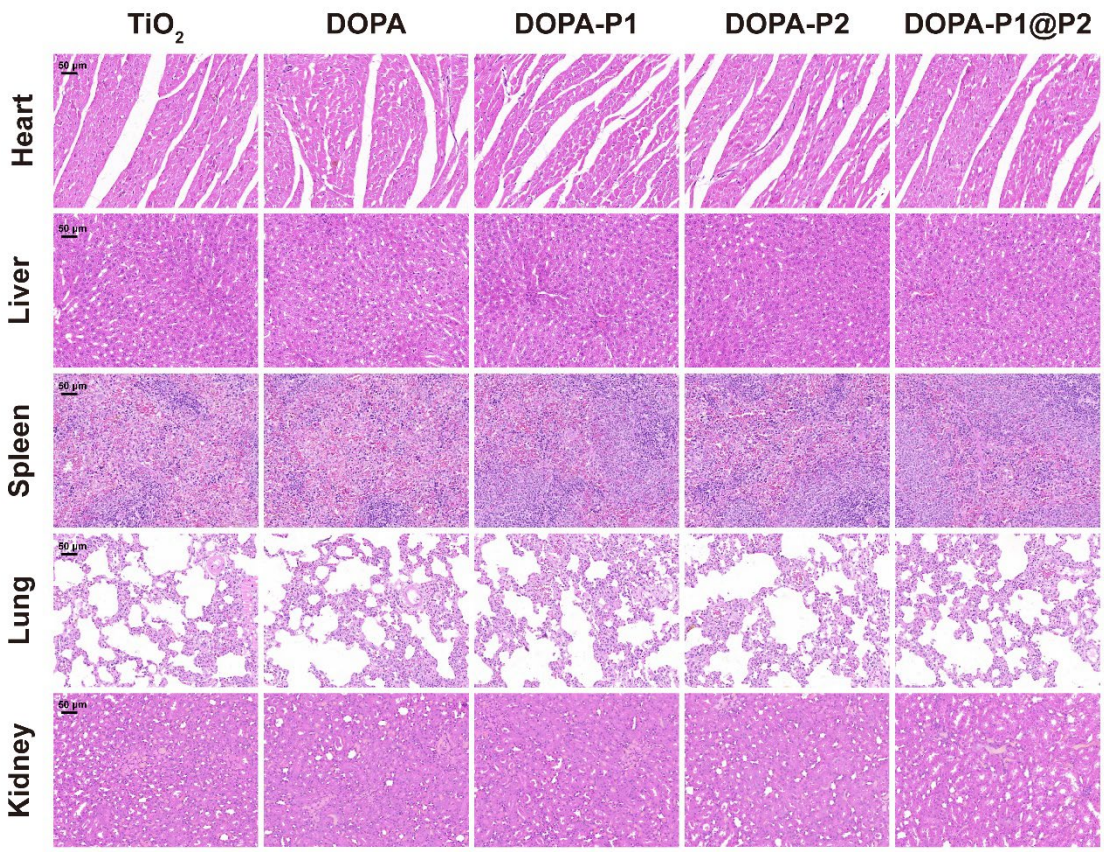
Supplementary Fig. 1 The molecular formula of the peptide and the synthesis process of (DOPA)$_4$-OEG$_5$-DBCO. (A–C) Molecular structures of (DOPA)$_4$-OEG$_5$-DBCO, P1 (N_3-K15-PVGLIG-K23), and P2 (N_3-Y5-PVGLIG-K23). **(D)** Synthesis process of (DOPA)$_4$-OEG$_5$-DBCO.



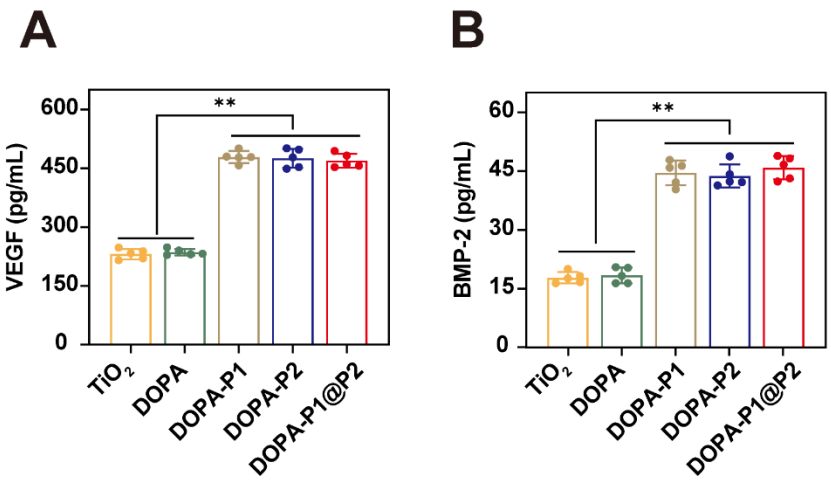
Supplementary Fig. 2 The synthesis process of P1 and P2. (A–B) Synthesis process of P1 (N_3-K15-PVGLIG-K23) and P2 (N_3-Y5-PVGLIG-K23).



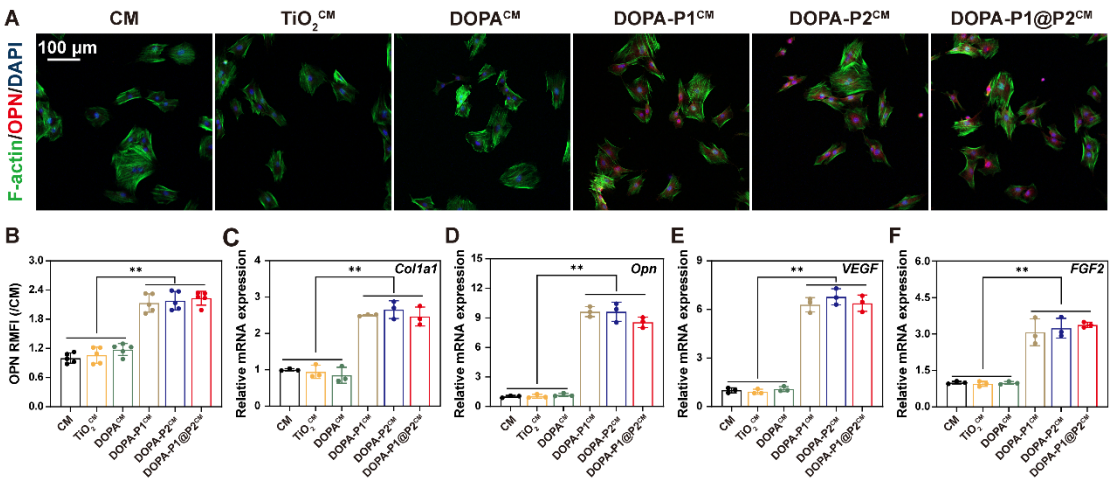
Supplementary Fig. 3 Exploration of the optimal ratio of P1 and P2. (A) Representative images of ALP staining and corresponding **(D)** quantitative analysis results after mixing P1 and P2 in different ratios (n=3 per group). **(B)** Representative images of ARS staining and corresponding **(E)** quantitative analysis results after mixing P1 and P2 in different ratios (n=3 per group). **(C)** Representative images of the tube formation assay and the corresponding **(F–G)** quantitative analysis results after mixing P1 and P2 in different ratios (n=3 per group). The data are presented as the mean ± standard deviation (SD). Data were analyzed by one-way ANOVA with Tukey's post hoc test (*$p < 0.05$, **$p < 0.01$ vs. the P1 : P2 = 4 : 0 group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. the P1 : P2 = 3 : 1 group; $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$ vs. the P1 : P2 = 2 : 2 group; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$ vs. the P1 : P2 = 1 : 3 group; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ vs. the P1 : P2 = 0 : 4 group). $p < 0.05$ and $p < 0.01$ indicate statistical significance. Exact p values were given in the Source Data file.



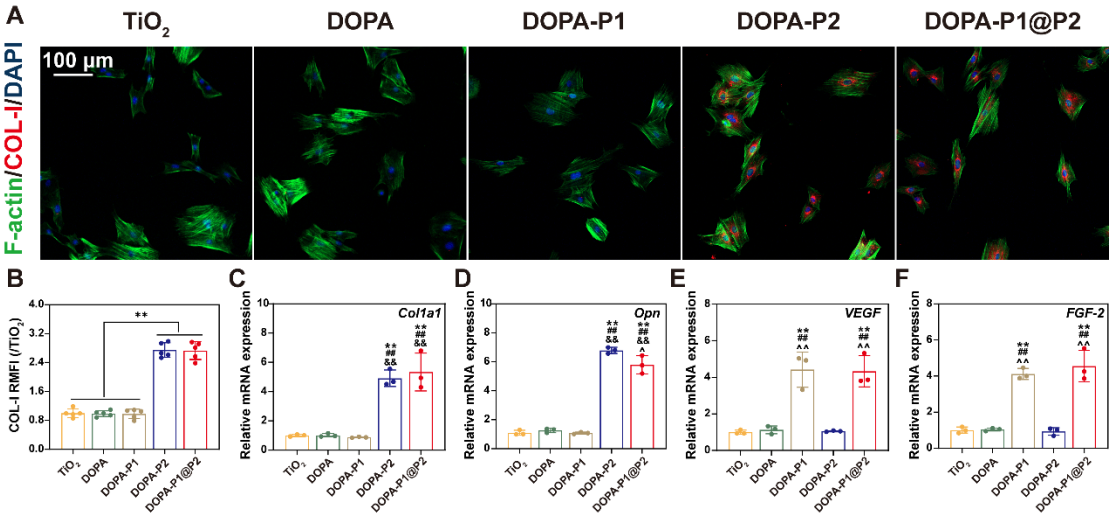
Supplementary Fig. 4: H&E staining of rat heart, liver, spleen, lung, and kidney at two months post-implantation.



Supplementary Fig. 5 Macrophage M2 polarization secretes cytokines that promote angiogenesis and osteogenesis. (A–B) ELISA quantified the secretion levels of the angiogenesis-related protein VEGF and the osteogenesis-related protein BMP-2. The data are presented as the mean ± SD ; n = 5 per group. Data were analyzed by one-way ANOVA with Tukey's post hoc test, and $*p < 0.05$ and $**p < 0.01$ indicate statistical significance. Exact p values were given in the Source Data file.



Supplementary Fig. 6 Supplementary experimental results on the indirect promotion of angiogenesis and osteogenesis. (A) Immunofluorescence staining evaluated the indirect osteogenic effects of modified surfaces, highlighting the cytoskeleton in green (F-actin), osteogenesis-related proteins in red (OPN), and nuclei in blue. **(B)** Quantitative results of immunofluorescence (n=5 per group). **(C–F)** RT-qPCR assessed the expression of osteogenic (*Colla1* and *Opn*) and angiogenic (*VEGF* and *FGF2*) genes to evaluate the indirect osteogenic effects of modified surfaces (n=3 per group). The data are presented as the mean ± SD. Data were analyzed by one-way ANOVA with Tukey's post hoc test, and $*p < 0.05$ and $**p < 0.01$ indicate statistical significance. Exact *p* values were given in the Source Data file.



Supplementary Fig. 7 Supplementary experimental results on the direct promotion of angiogenesis and osteogenesis. (A) Immunofluorescence staining evaluated the direct osteogenic effects of modified surfaces, highlighting the cytoskeleton in green (F-actin), osteogenesis-related proteins in red (COL-I), and nuclei in blue. **(B)** Quantitative results of immunofluorescence (n=5 per group). **(C–F)** RT-qPCR assessed the expression of osteogenic (*Colla1* and *Opn*) and angiogenic (*VEGF* and *FGF2*) genes to evaluate the direct osteogenic effects of modified surfaces (n=3 per group). The data are presented as the mean ± SD. Data were analyzed by one-way ANOVA with Tukey's post hoc test ($^{*}p < 0.05$ and $^{**}p < 0.01$ vs. the TiO_2 group or comparisons between combined groups indicated in the figure; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ vs. the DOPA group; $^{\&}p < 0.05$ and $^{\&\&}p < 0.01$ vs. the DOPA-P1 group; $^{\wedge}p < 0.05$ and $^{\wedge\wedge}p < 0.01$ vs. the DOPA-P2 group). $p < 0.05$ and $p < 0.01$ indicate statistical significance. Exact p values were given in the Source Data file.

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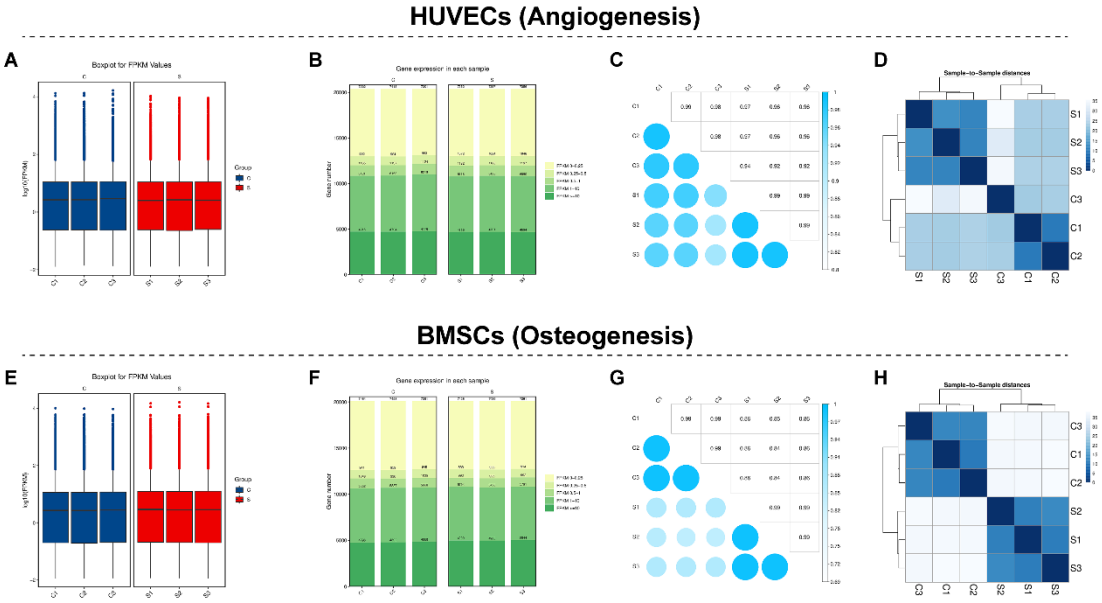

Supplementary Fig. 8 Supplementary quality control data for transcriptome sequencing. **(A, E)** Box plot of gene expression levels. **(B, F)** Area distribution map of gene expression levels. **(C, G)** Heatmap of correlation coefficients between samples. **(D, H)** Results of sample-to-sample cluster analysis. Statistical analysis was performed by unpaired two-tailed Student's *t*-test.

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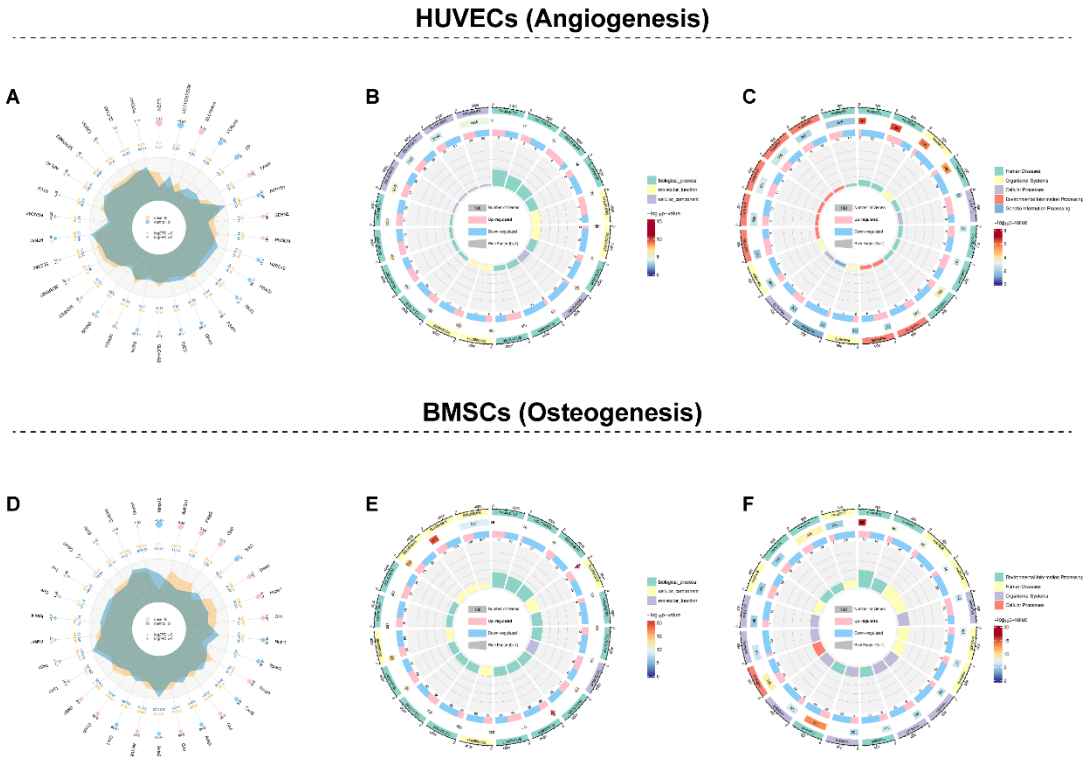
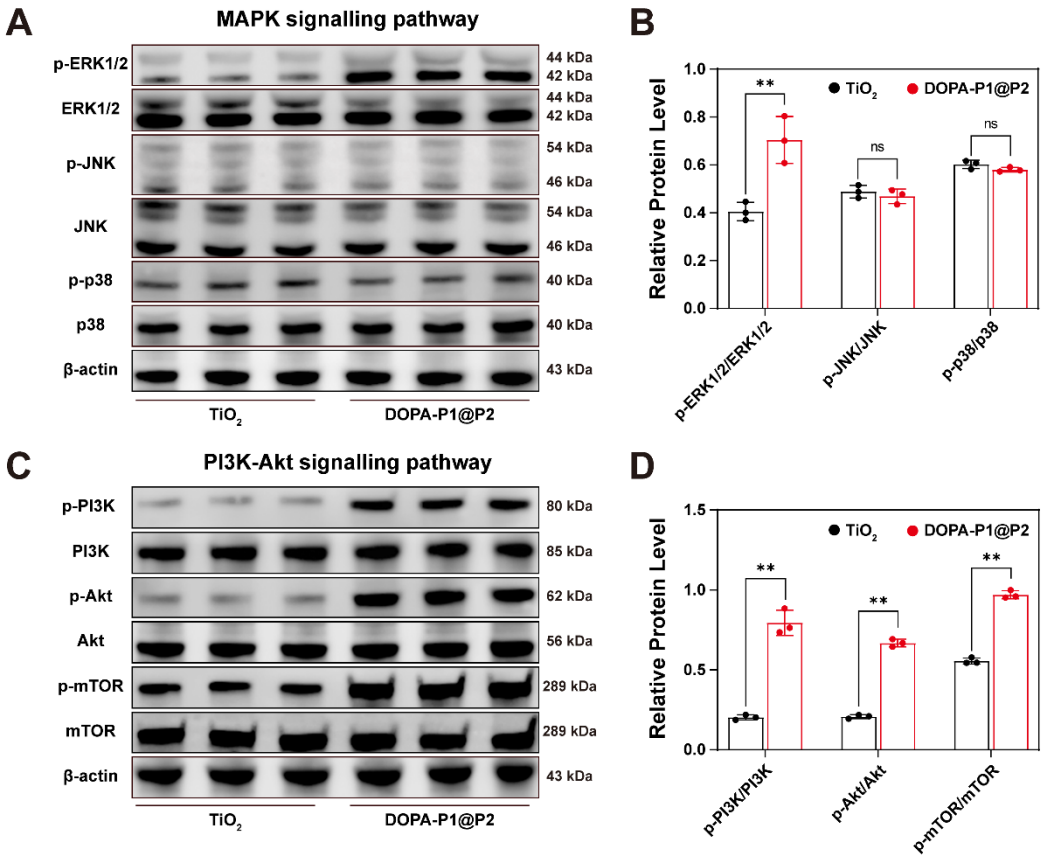
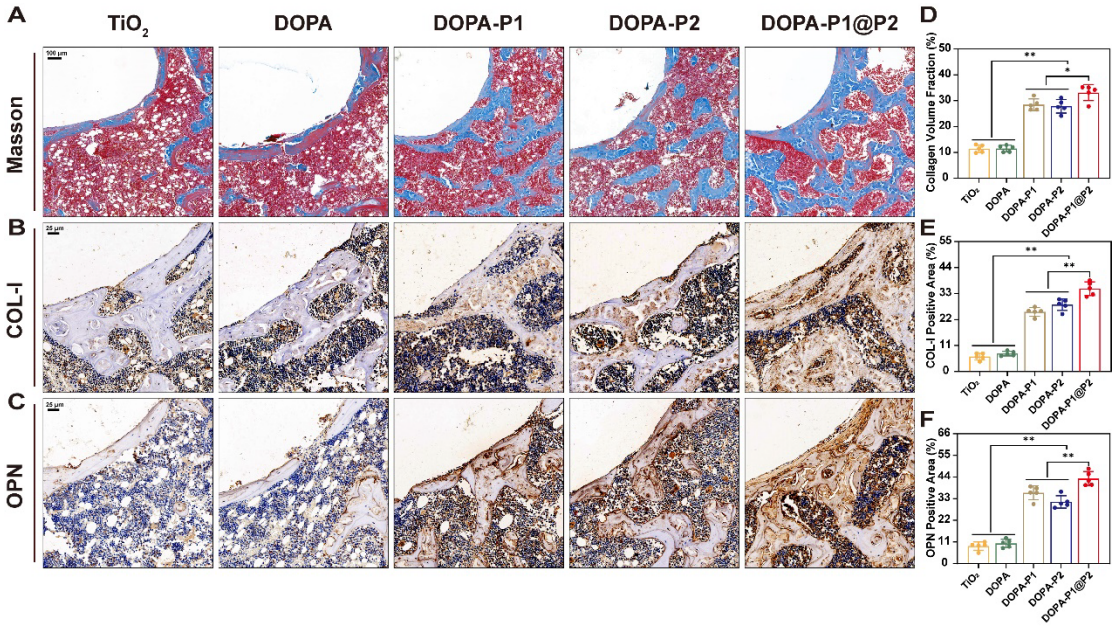


Supplementary Fig. 9 Supplementary GO and KEGG data for transcriptome sequencing. **(A, D)** Differential gene radar chart. **(B, E)** Circle plot of Gene Ontology (GO) enrichment analysis for differentially expressed genes. **(C, F)** Circle plot of Encyclopedia of Genes and Genomes (KEGG) enrichment analysis for differentially expressed genes. Statistical analysis was performed by unpaired two-tailed Student's *t*-test.



Supplementary Fig. 10 Validation of DOPA-P1@P2's mechanism in promoting angiogenesis and osteogenesis. (A–B) WB detected the expression levels of MAPK signalling pathway-related proteins, along with the corresponding quantitative analysis results (n=3 per group). **(C–D)** WB detected the expression levels of PI3K–AKT signalling pathway-related proteins, along with the corresponding quantitative analysis results (n=3 per group). The data are presented as the mean ± SD. Statistical analysis was performed by unpaired two-tailed Student's *t*-test., and $*p < 0.05$ and $**p < 0.01$ indicate statistical significance. Exact *p* values were given in the Source Data file.



Supplementary Fig. 11: Supplementary in vivo experimental results on promoting osseointegration. (A, D) Masson's staining and corresponding quantitative analysis results (n=5 per group). **(B–C)** Immunohistochemical staining assessed the expression of osteogenic proteins (COL-I and OPN), accompanied by corresponding **(E–F)** quantitative analysis (n=5 per group). The data are presented as the mean ± SD. Data were analyzed by one-way ANOVA with Tukey's post hoc test, and $*p < 0.05$ and $**p < 0.01$ indicate statistical significance. Exact *p* values were given in the Source Data file.

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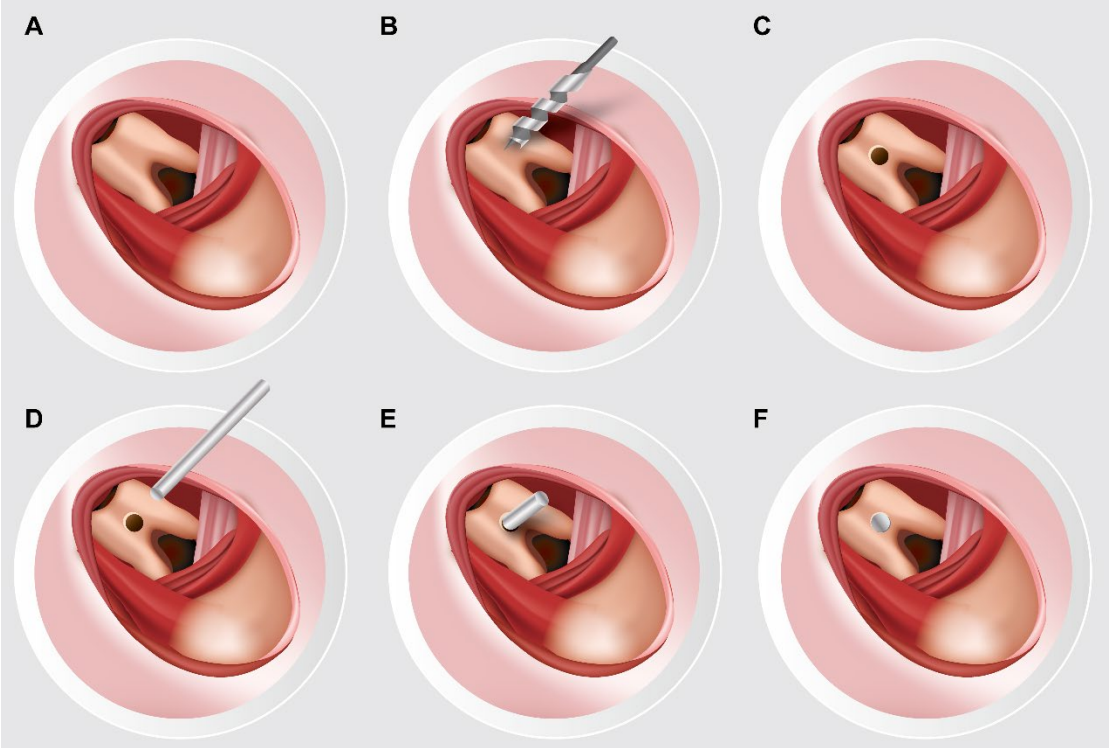

Supplementary Fig. 12 Schematic diagram of the animal model surgery. (A) Exposure of the femur condyles **(B)** Drilling of the femur condyles **(C)** Completion of the drilling process **(D–E)** Implantation of the titanium rod **(F)** Completion of the implantation process

Supplementary Information

Supplementary Table 1. Primer sequences used in RT-PCR analysis.

Genes	Primes (F-Forward, R-Reverse, 5‘-3’)
Mouse-*Tnf*	F: GTTCCCAAATGGCCTCCC
	R: GTGCTCCTCACCCACACCG
Mouse-*Il6*	F: GAGACCACTGGGGAGAATGC
	R: TTGCCAGGTGGGTAAAGTGG
Mouse-*Il10*	F: CCCTTTGCTATGGTGTCCT
	R: GTGGCCAGTTTGTTATTTAT
Mouse-*Ccr7*	F: GGTGGCTCTCCTTGTCATTTTC
	R: AGGTTGAGCAGGTAGGTATCCG
Mouse-*Cd206*	F: TACTTGGACGGATAGATGGAGG
	R: CATAGAAAGGAATCCACGCAGT
Mouse-*Bmp2*	F: AACGAGAAAAGCGTCAAGCC
	R: AGGTGCCACGATCCAGTCAT
Mouse-*Vegf*	F: AGGAGTCCCCGACGAGATAGA
	R: CACATCTGCTGTGCTGTAGGAA
Mouse-*Actb*	F: GTGACGTTGACATCCGTAAAGA
	R: GTAACAGTCCGCCTAGAAGCAC
Rat-*Col1a1*	F: AGCTCGATACACAATGGCCT
	R: CCTATGACTTCTGCGTCTGG
Rat-*Opn*	F: GAACATGAAATGCTTCTTTCTCAG
	R: TCCATGAAGCCACAAACTAAACTA
Rat-*Actb*	F: CCTCTATGACAACACAGT
	R: AGCCACCAATCCACACAG
Human-*VEGF*	F: TATGCGGATCAAACCTCACCA
	R: CACAGGGATTTTCTTGTCTTGCT
Human-*FGF2*	F: AAGAGCGACCCTCACATCAA
	R: GCCAGGTAACGGTTAGCACA
Human-*GADPH*	F: CATCATCCCTGCCTCTACTGG
	R: GTGGGTGTCGCTGTTGAAGTC