Supporting Information

Synergistic Effect of ROS-Generating Polydopamine on Drug-Induced Bone Tissue Regeneration

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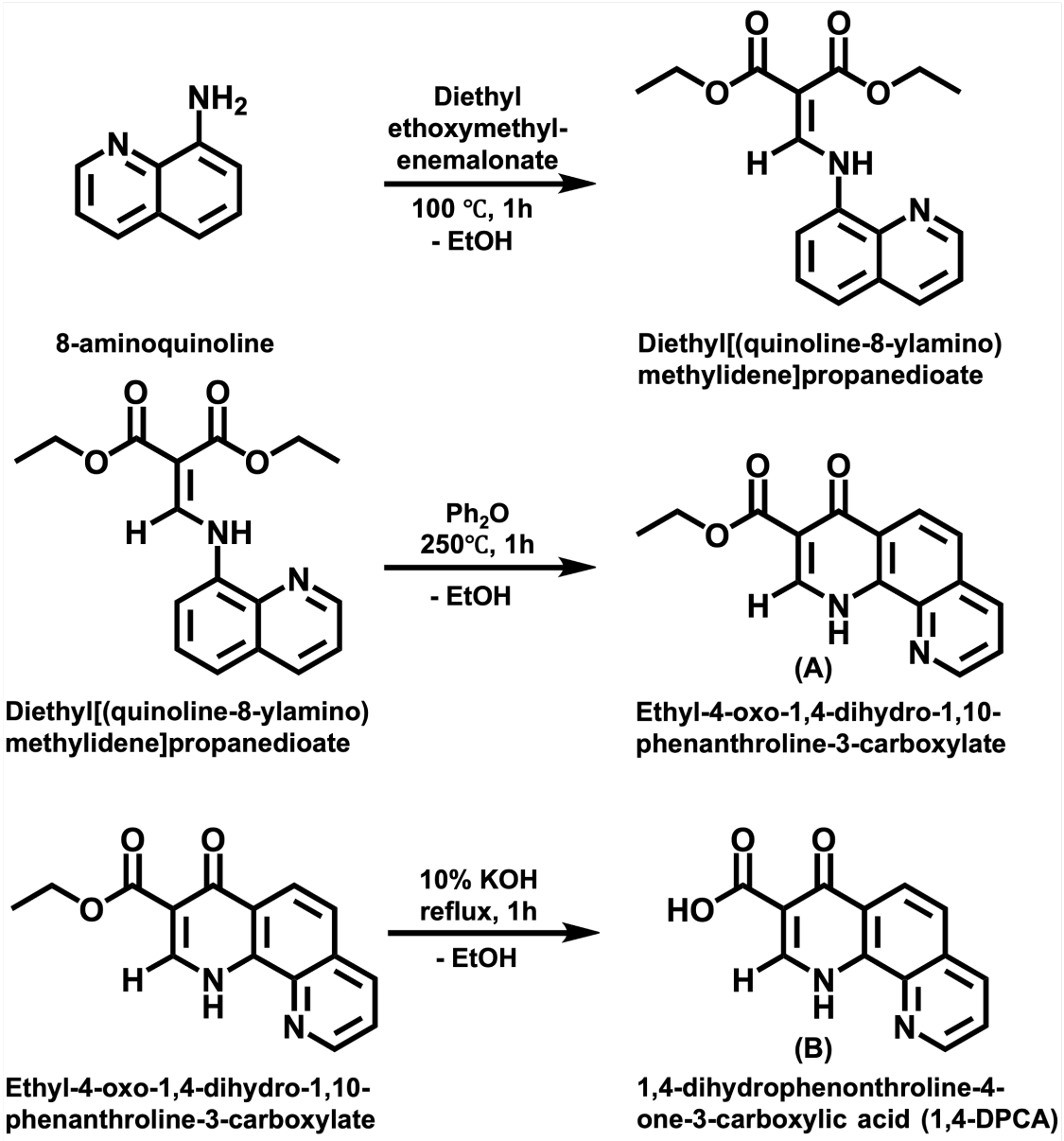

Fig. S1. Synthetic scheme of 1,4-DPCA. The synthesis was accomplished in two steps, and the two main products (A, B) are described here.

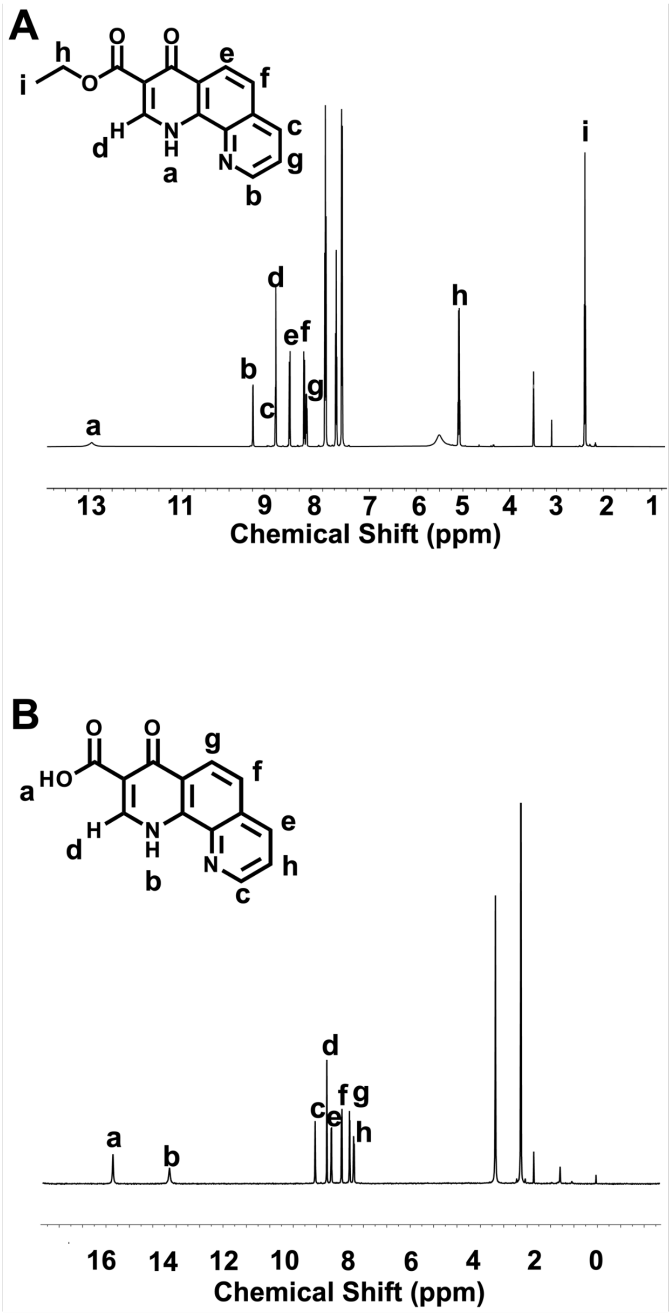

Fig. S2. (A) The intermediate compound, ethyl-4-oxo-1,4-dihydro-1,10-phenanthroline-3-carboxylate. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.83 (d, J = 6.6 Hz, 1H), 9.06 (dd, J = 4.3, 1.7 Hz, 1H), 8.57 – 8.50 (m, 2H), 8.22 (d, J = 8.7 Hz, 1H), 7.86 (d, J = 8.9 Hz, 1H), 7.80 (dd, J = 8.3, 4.3 Hz, 1H), 4.25 (q, J = 7.1 Hz, 2H), 1.29 (t, J = 7.1 Hz, 3H). (B) 1,4-DPCA. ^{1}H NMR (500 MHz, DMSO-d_6) δ 15.44 (s, 1H), 13.88 (s, 1H), 9.18 (dd, J = 4.2, 1.6 Hz, 1H), 8.77 (d, J = 6.4 Hz, 1H), 8.66 (dd, J = 8.3, 1.6 Hz, 1H), 8.31 (d, J = 8.8 Hz, 1H), 8.08 (d, J = 8.8 Hz, 1H), 7.94 (dd, J = 8.2, 4.2 Hz, 1H).

Fig. S3. Optical image demonstrating the Tyndall effect exhibited in the solution containing 1,4-DPCA aggregates.

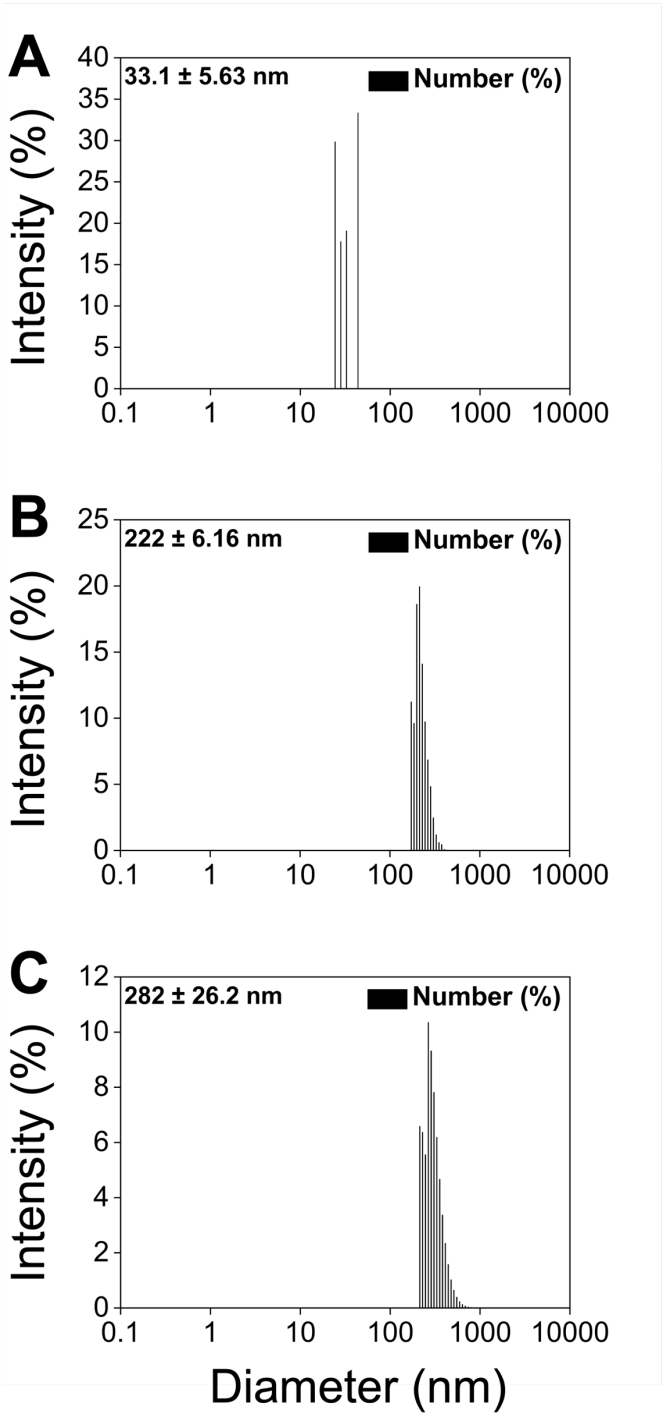

Fig. S4. Particle diameter distribution of (A) 1,4-DPCA drug aggregates, (B) polydopamine nanoparticles (PDA NPs), and (C) polydopamine nanoparticles loaded with 1,4-DPCA (PDA w/DPCA NPs).

	Case 1	Case 2
DDS Structure	Randomly-Distributed	Core-Shell
Theoretical Value	[at% of -NH-] PDA NPs = 58 at% 1,4-DPCA = 25% DDS = (0.9 x 58) + (0.1 x 25) = **54.7 at%**	[at% of -NH-] PDA NPs = 58 at% DDS = (1.0 x 58) = **58 at%**
Experimental Value	**57 at%**	

Table S1. Comparison of theoretical and experimental atomic percentages of the -NH- bond for randomly distributed and core-shell structured DDS.

	Case 1	Case 2
DDS Structure	Randomly-Distributed	Core-Shell
Theoretical Value	[at% of O-C] PDA NPs = 8 at% 1,4-DPCA = 41% DDS = (0.9 x 8) + (0.1 x 41) = **11.3 at%**	[at% of O-C] PDA NPs = 8 at% DDS = (1.0 x 8) = **8 at%**
Experimental Value	**7 at%**	

Table S2. Comparison of theoretical and experimental atomic percentages of the O-C bond for randomly distributed and core-shell structured DDS.

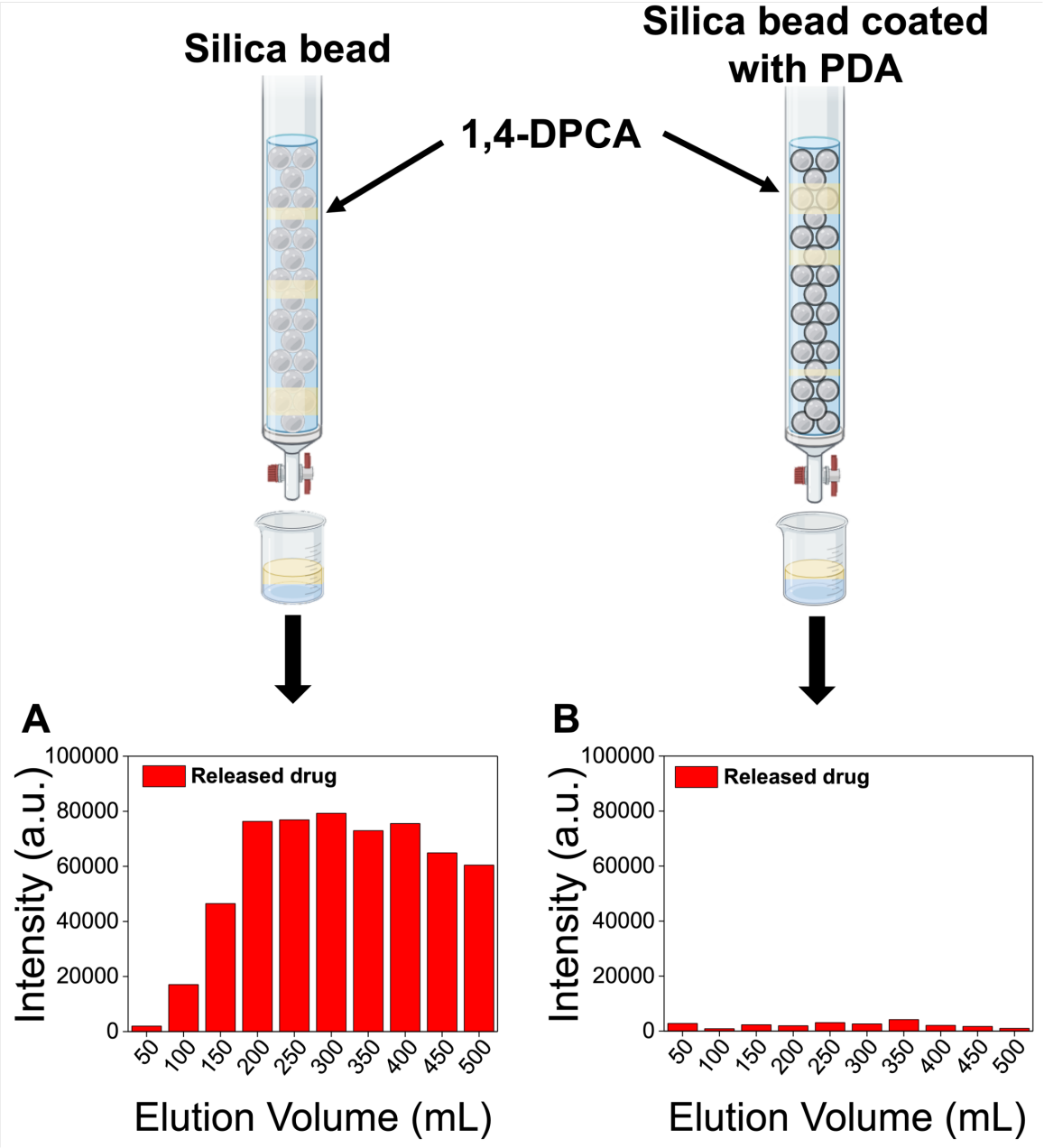

Fig. S5. The amount of drug released from a column filled with (A) silica beads and (B) silica beads coated with PDA was measured by high-performance liquid chromatography. a.u. indicates arbitrary unit.

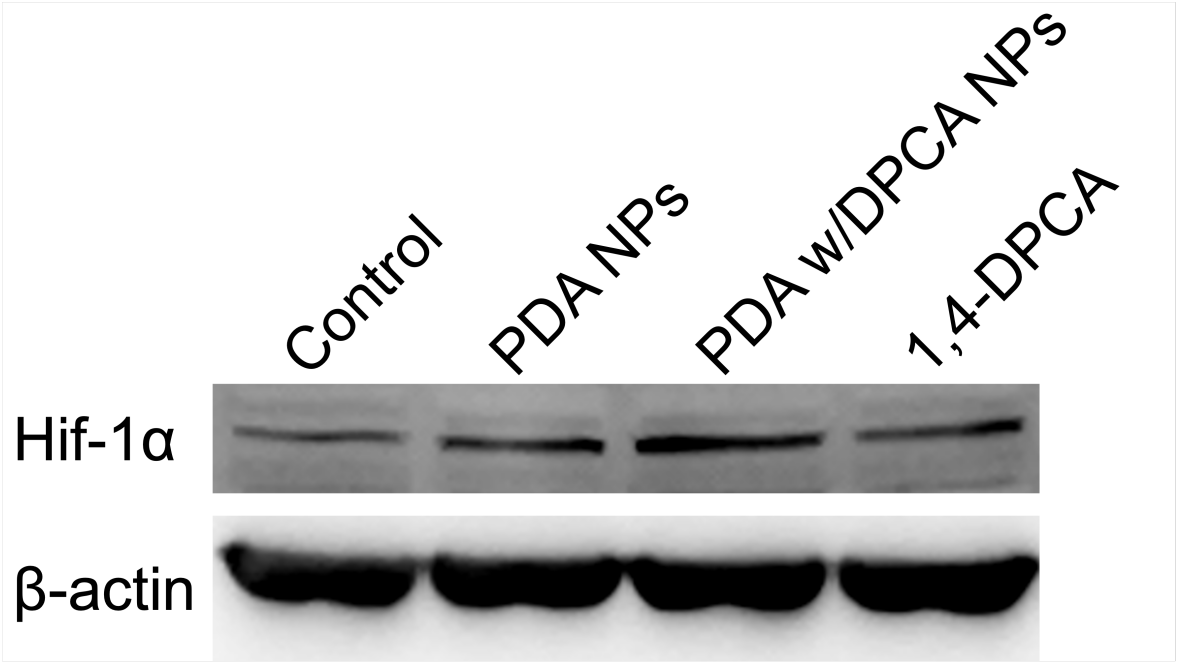

Fig. S6. Western blot analysis showing the expression levels of Hif-1α in different treatment groups. MC3T3-E1 cells were treated with control (untreated), PDA NPs, PDA w/DPCA NPs, and 1,4-DPCA, as indicated. Hif-1α levels were detected using specific antibodies, with β-actin used as a loading control.

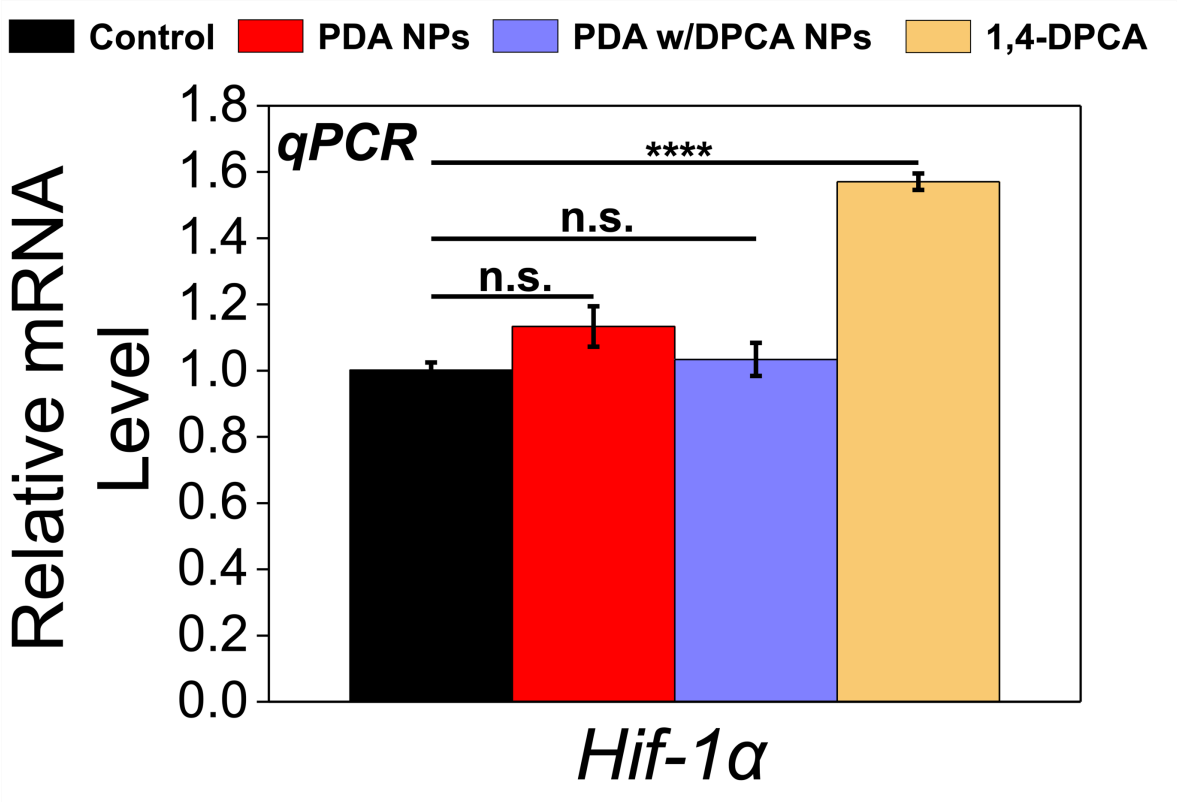

Fig. S7. Relative *Hif-1α* mRNA expression in MC3T3-E1 cells treated with PDA NPs, PDA w/DPCA NPs, and 1,4-DPCA, assessed using RT-qPCR (n = 6). Asterisks indicate statistical significance (p-values): **** represents $p \leq 0.0001$, and n.s. = not significant.

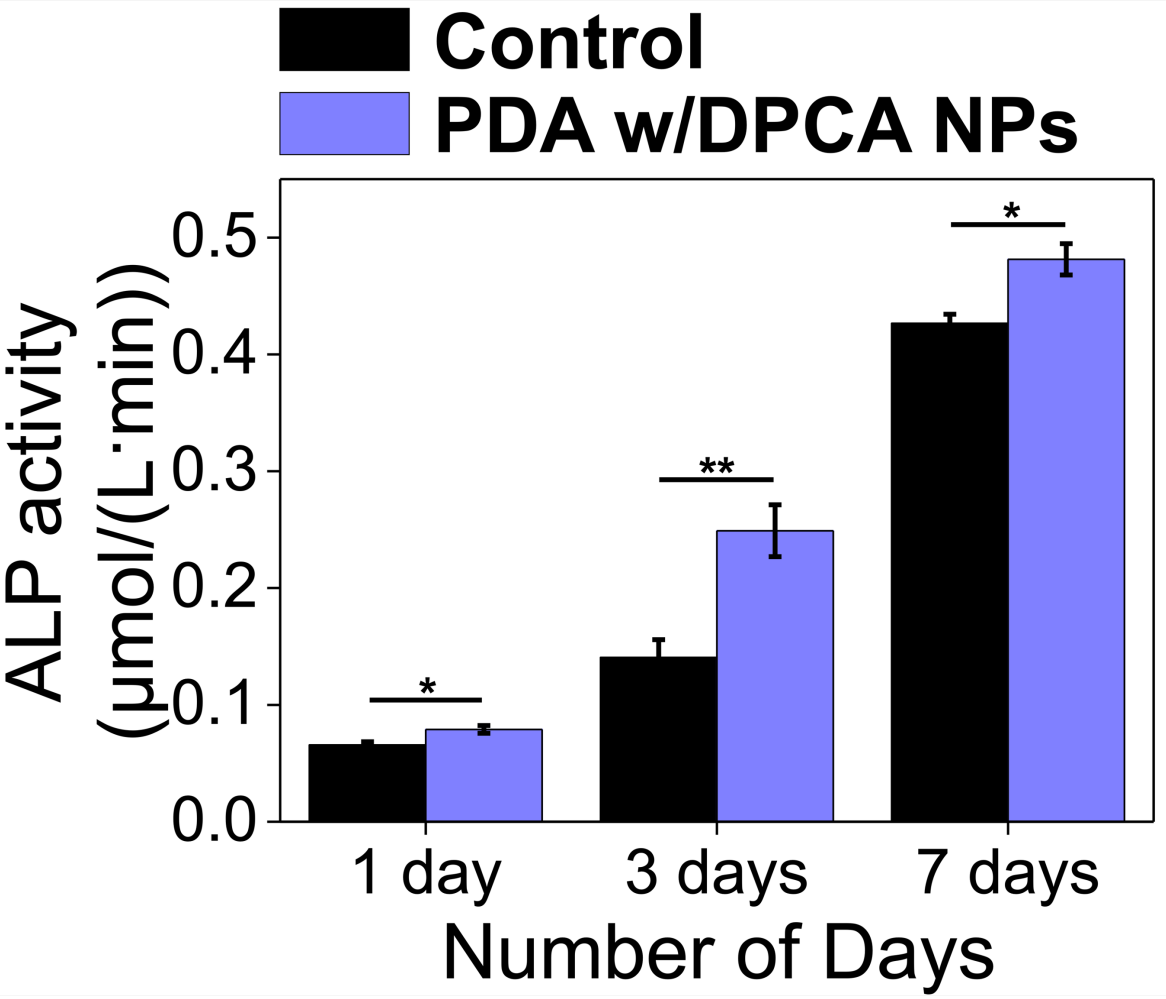

Fig. S8. Alkaline phosphatase (ALP) activity assay: reported values are presented as the mean ± SD, and the assay was performed after 1, 3, and 7 days of culture (n = 4). The area of tissue regeneration is indicated by a black dotted line. *p ≤ 0.05, **p ≤ 0.01, n.s. = not significant.

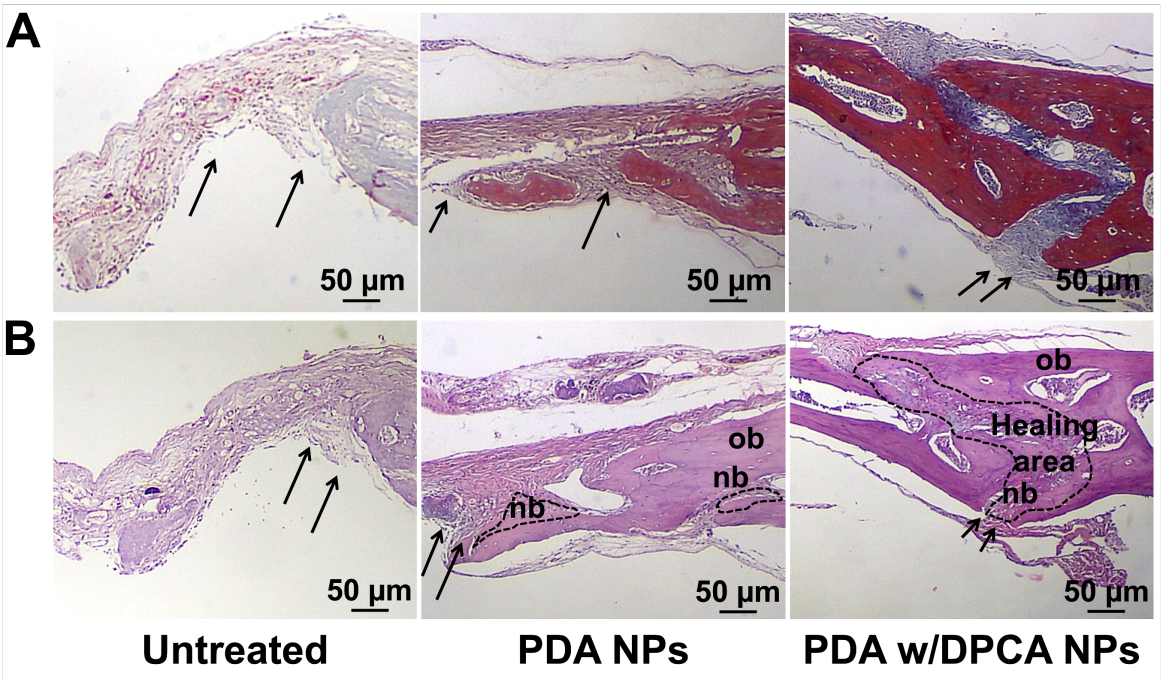

Fig. S9. (A) Representative MT staining images of the calvarial bone defect sections from each group. Scale bar = 50 μm. (B) Representative H&E staining images of calvarial bone defect sections from animals in the untreated, and PDA NPs and PDA w/DPCA NPs treated groups. The arrow sign shows the defect site; “ob” indicates old bone, and “nb” indicates new bone.