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Supplementary Content

Surface Modification of Titanium with Curcumin: A Promising Strategy to Combat Fibrous Encapsulation

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Table S1 Primer sequences used in this study.

Gene	Primer Sequence
Mouse *Acta2*	5’-ATCGTCCACCGCAAATGC-3’(forward)
	5’-AAGGAACTGGAGGCGCTG-3’(reverse)
Mouse *Col1a1*	5’-TGAGACAGGCGAACAAGG-3’(forward)
	5’-CAGGAGAACCAGCAGAGC-3’(reverse)
Mouse *Fn1*	5’-CTGTGACAACTGCCGTAG-3’(forward)
	5’-CAGCTTCTCCAAGCATCG-3’(reverse)
Mouse *Gapdh*	5’-GCACAGTCAAGGCCGAGAAT-3’(forward)
	5’-GCCTTCTCCATGGTGGTGAA-3’(reverse)

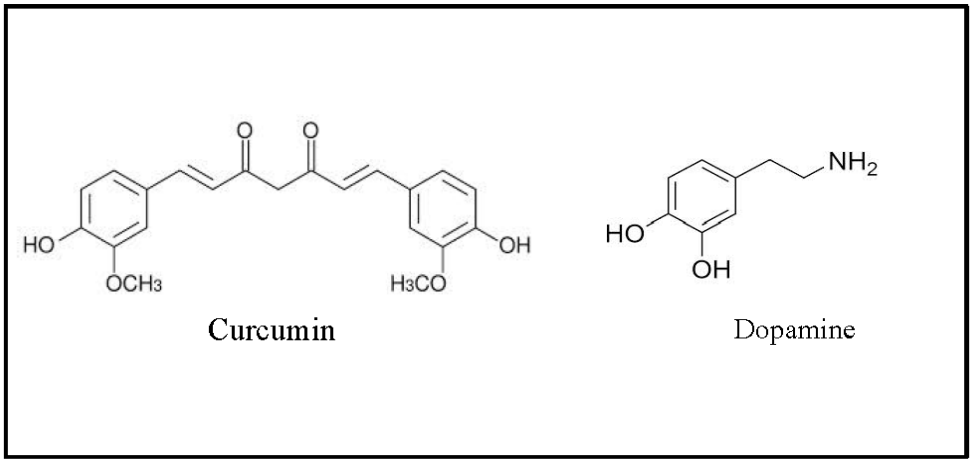

Fig. S1 Chemical structures of curcumin and dopamine.

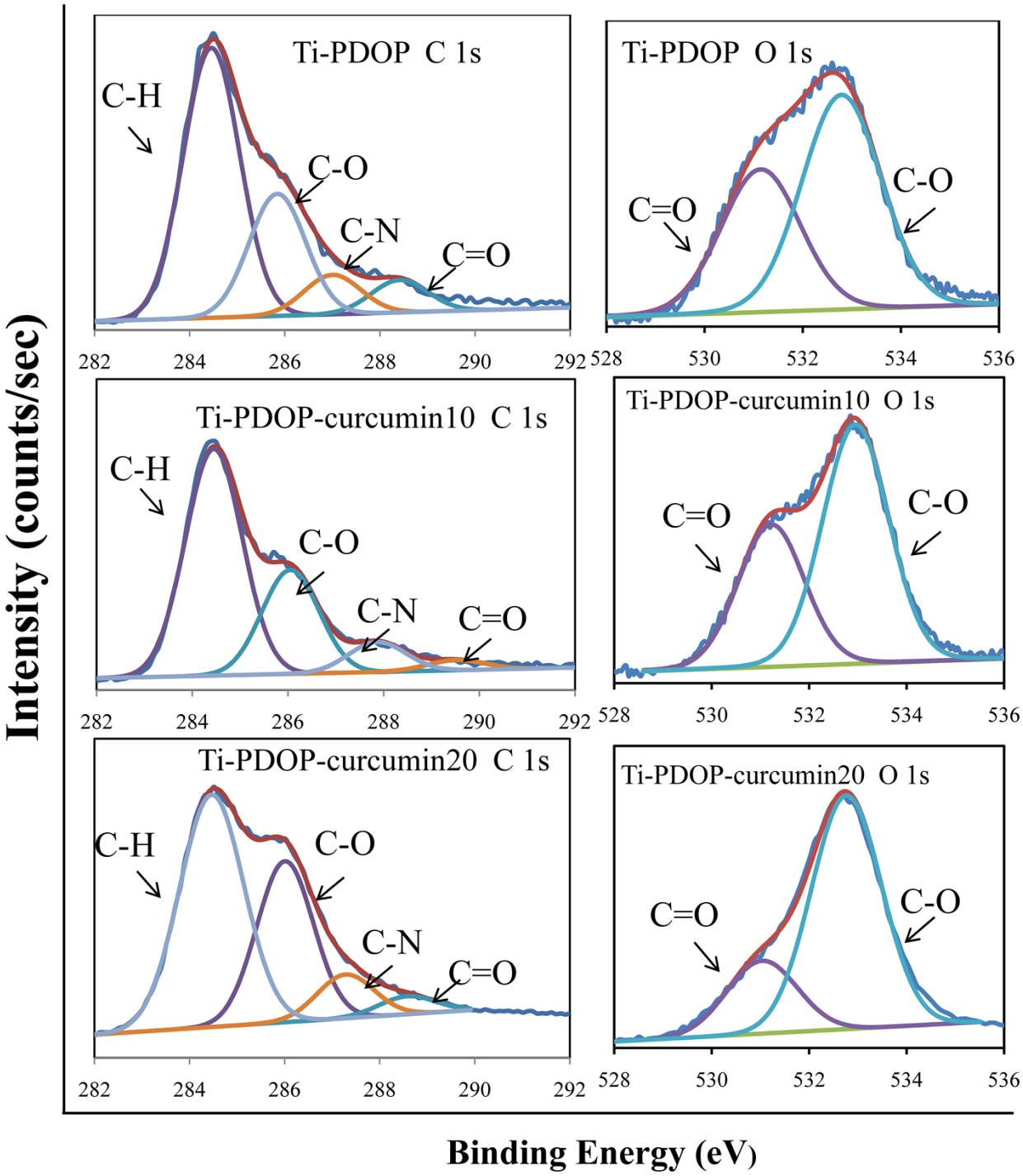

Fig. S2 C 1s and O 1s core-level XPS spectra of the Ti-PDOP, Ti-PDOP-curcumin10, and Ti-PDOP-curcumin20 substrates. Immobilization of curcumin on the polydopamine-modified surface resulted in an increase in the C-O content and a reduction in the C=O content, possibly due to the higher level of C-O in curcumin than dopamine (Fig. S1).

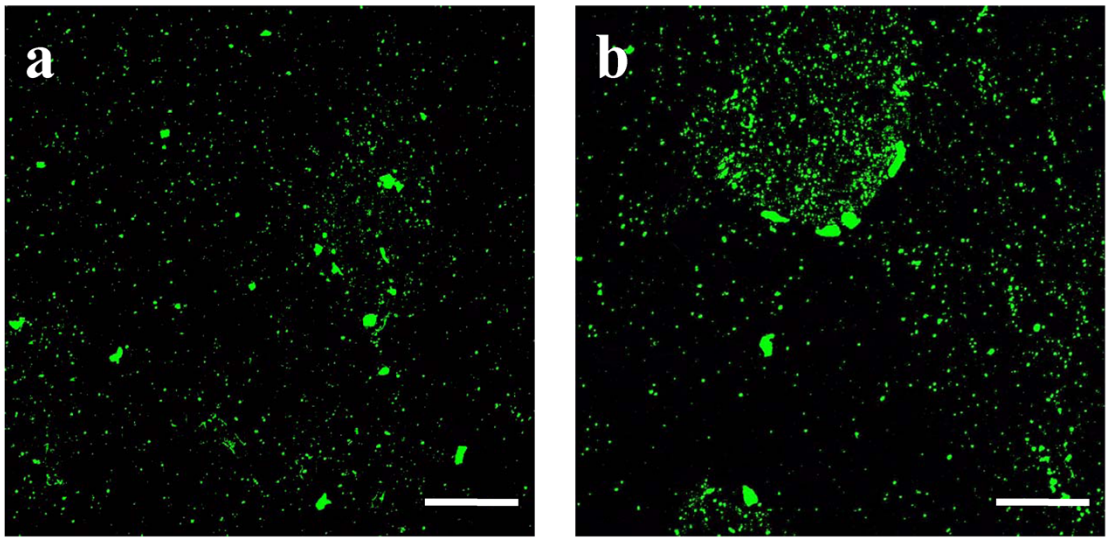

Fig. S3 CLSM images of (a) Ti-PDOP-curcumin10 and (b) Ti-PDOP-curcumin20 substrates after immerged in PBS for 7 days (treated with HP-β-CD). Scale bar = 500 μm.

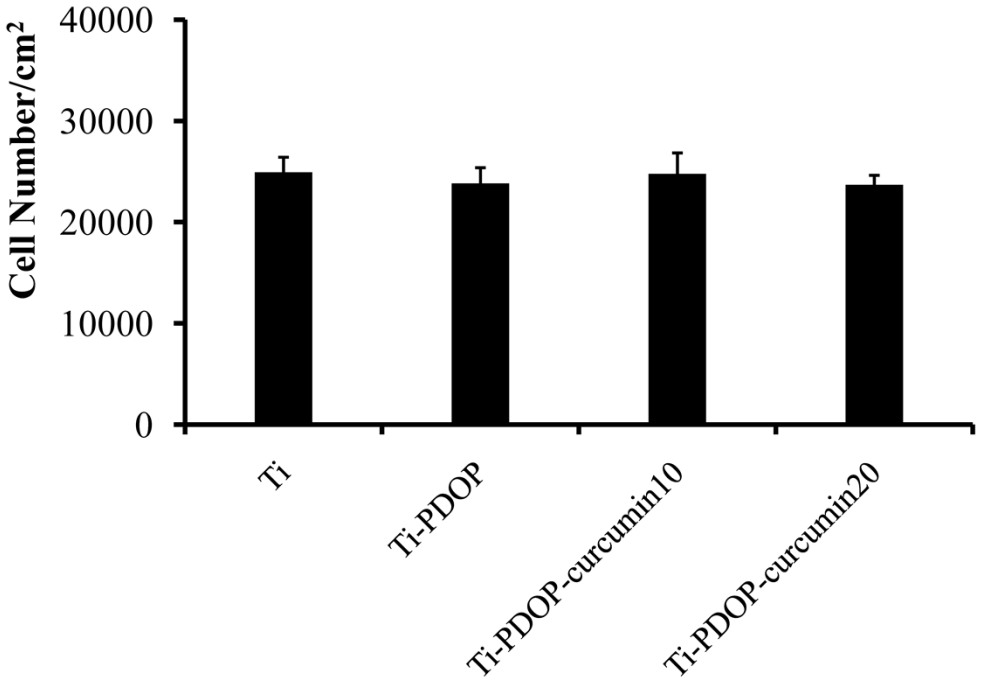

Fig. S4 The numbers of attached fibroblasts on Ti, Ti-PDOP, Ti-PDOP-curcumin10, and Ti-PDOP-curcumin20 substrates. No significant difference was observed between the curcumin-functionalized Ti and Ti substrates.

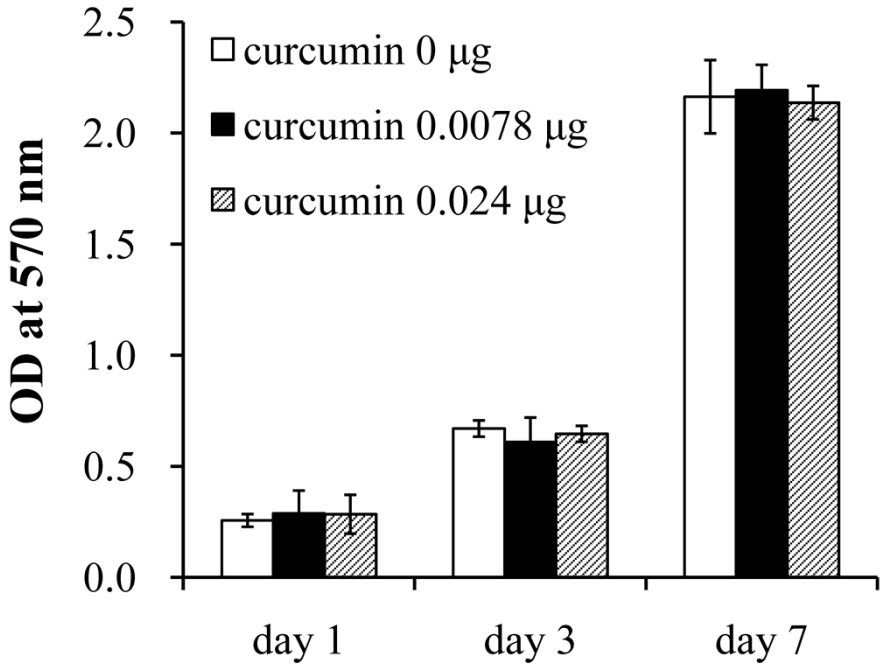

Fig. S5 MTT results of the released curcumin on fibroblasts. Cells were cultured on a 24-well microplate for 7 days in growth medium supplemented with different amount of curcumin: 0 μg, 0.0078 μg (the amount of curcumin released from the Ti-PDOP-curcumin10 substrate after incubation in PBS for 48 h) and 0.024μg (the amount of curcumin released from the Ti-PDOP-curcumin20 substrate after incubation in PBS for 48 h) (n=3). No significant difference was observed between groups treated with or without curcumin on day 1, 3 and 7.

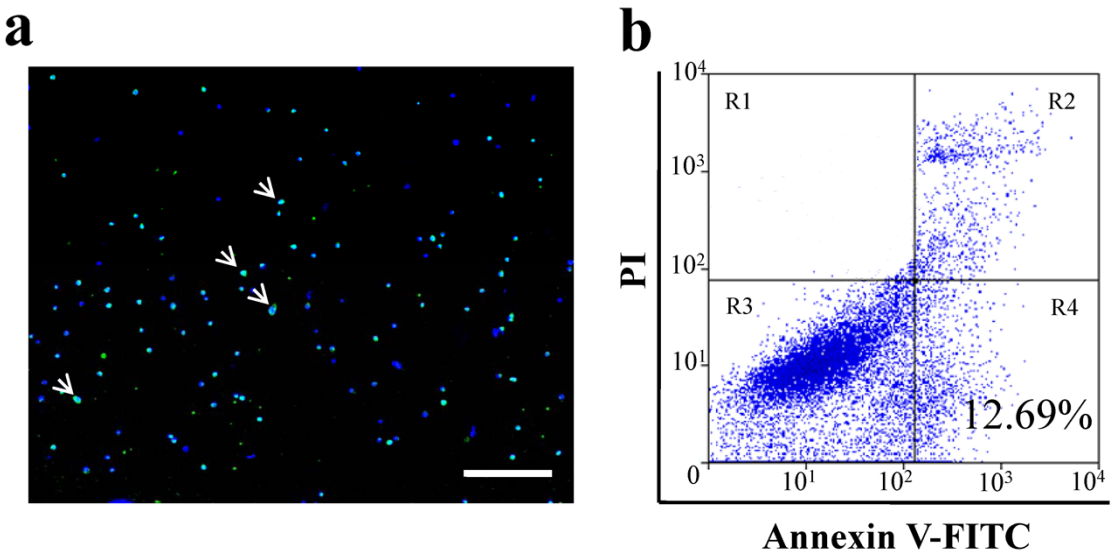

Fig. S6 (a) Fluorescence microscopy images of fibroblasts on the Ti substrate after culturing for two days followed by incubation with 10 μM camptothecinin in growth medium for 4 h in 37 ℃.All the nuclei exhibited blue fluorescence, and the appearance of green fluorescence (as marked with white arrows) indicated apoptosis. Scale bar = 500 μm.(b) Flow cytometry analysis of fibroblasts after culturing on Ti for two days and incubation with 10 μM camptothecinin in growth medium for 4 h in 37 ℃ followed by staining with Annexin V-FITC/PI. In each section, the left upper quadrant (R1) and the right upper quadrant (R2) represented necrotic cells and dead cells, respectively; the left lower quadrant (R3) and the right lower quadrant (R4) represented live cells and apoptosis cells (marked with percentage), respectively.

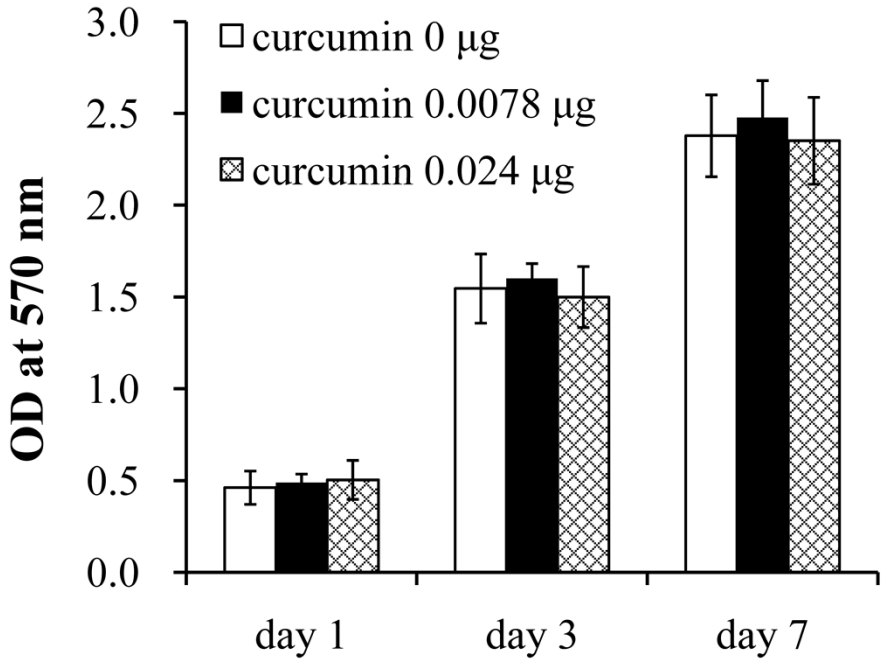

Fig. S7 MTT results of the released curcumin on osteoblasts. Cells were cultured on a 24-well microplate for 7 days in growth medium supplemented with different amount of curcumin: 0 μg, 0.0078 μg (the amount of curcumin released from the Ti-PDOP-curcumin10 substrate after incubation in PBS for 48 h) and 0.024 μg (the amount of curcumin released from the Ti-PDOP-curcumin20 substrate after incubation in PBS for 48 h) (n=3). No significant difference was observed between groups treated with or without curcumin on day 1, 3 and 7.

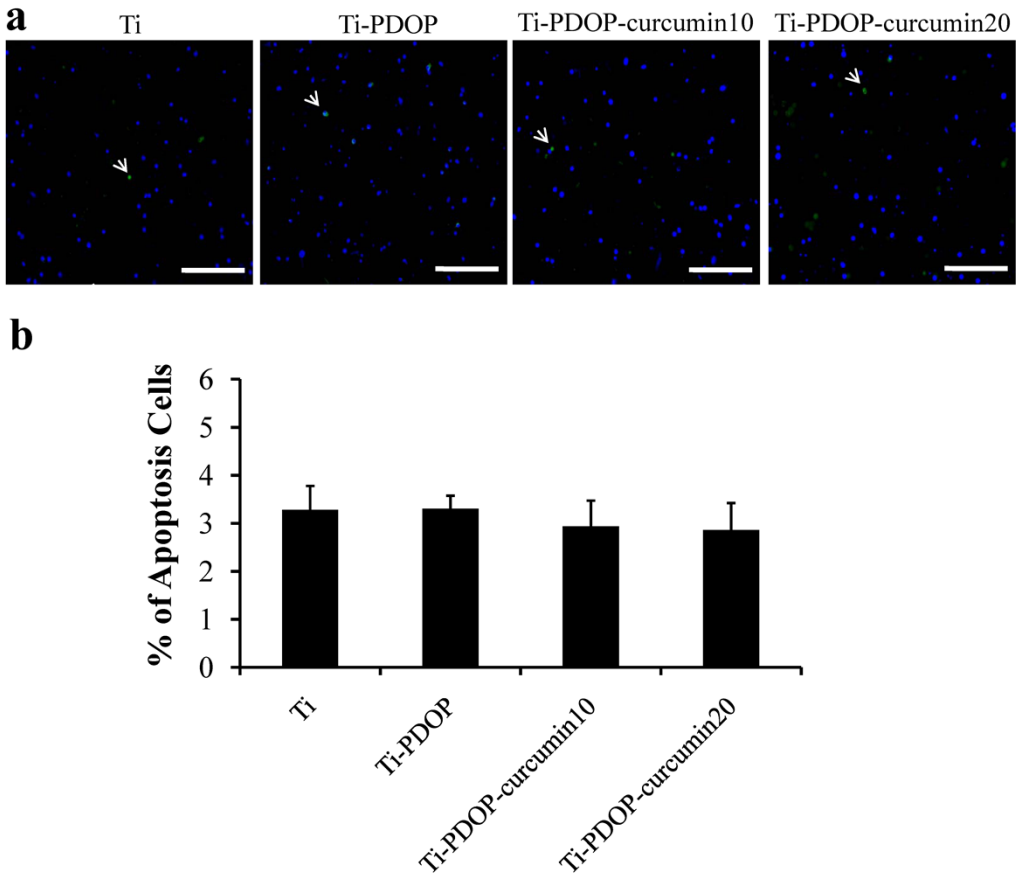

Fig. S8 Apoptosis of osteoblasts on Ti, Ti-PDOP, Ti-PDOP-curcumin10 and Ti-PDOP-curcumin20 substrates 48 h after cell seeding: (a) Fluorescence microscopy images of fibroblasts on the pristine and functionalized Ti substrates. All the nuclei exhibited blue fluorescence, and the appearance of green fluorescence (as marked with white arrows) indicated apoptosis. Scale bar = 500 μm. (b) Percentages of apoptotic cells on the pristine and functionalized Ti substrates determined by flow cytometry analysis (n=3).