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A novel Coating with universal adhesion and inflammation-responsive drug release functions to manipulate osteoimmunomodulation of implants

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1.1 *In vitro* Biocompatibility Evaluation

Raw264.7 macrophage cells and rBMSCs were used to evaluate the in vitro biocompatibility of the coating. 1×10^4 cells were seeded on each samples. After culturing for 1, 3, and 5 days, the cells were rinsed with PBS buffer and incubated in 10% CCK-8 containing medium for another 4 h at 37 °C, and then the absorbance at 450 nm was acquired by a spectrophotometer. For cell morphology observation, the cells were dehydrated by graded ethanol and then dried in hexamethyldisilane. Finally, the cell morphology was observed by SEM after sputtered with gold.

1.2 Gene Expression of Macrophage on the Coatings.

Gene expression of macrophages on the coatings. 1.5×10^5 cells were seeded onto the coatings. After culturing for 4 days, the cell seeded samples were washed twice with PBS and total RNA from the cells were extracted using TRIzol reagent (Life Technologies), and were used to synthesize complementary DNA using the DyNAmo™ cDNA Kit following the manufacturer's instructions. RT-PCR assay was applied to detect the expression of inflammatory genes (IL-1β, IL-6, TNF-α， IL-4, IL-10), macrophage phenotype markers (CD86 for M1, CD-206 and CD163 for M2), osteogenic-related genes (BMP-2, TGF-β1, VEGF), and osteoclastic-related genes (TRAP and STSK). RAW264.7 cells cultured in the 6-well plate were used as control.

Cytokines expressions of macrophages on the coatings. 1.5×10^5 cells were seeded onto the coatings. After 12 h culturing, the normal medium was replaced by medium with 1 μg/mL lipopolysaccharide (LPS) to activate inflammatory macrophages. After 2 h stimulation, the medium was replaced by serum-free culture medium for another 6 h culturing. Then, the supernatant was collected and centrifuged, and ELISA kits were used to examine the concentrations of IL-1β, IL-4, IL-6, IL-10, and TNF-α.

1.3 OIM property evaluation of the coatings

To further confirm its osteogenic differentiation potential, alizarin Red staining was used to highlight mineralization nodules in BMSCs grown in a 96-well plate for 14 days in conditioned medium with osteogenic components. The cells were washed with distilled water and fixed in 4% paraformaldehyde for 10 min. After rinsing with distilled water, the cells were stained in a solution of 2% Alizarin Red S at pH 4.1 for 20 min. The samples were air-dried, and images acquired with a light microscope.

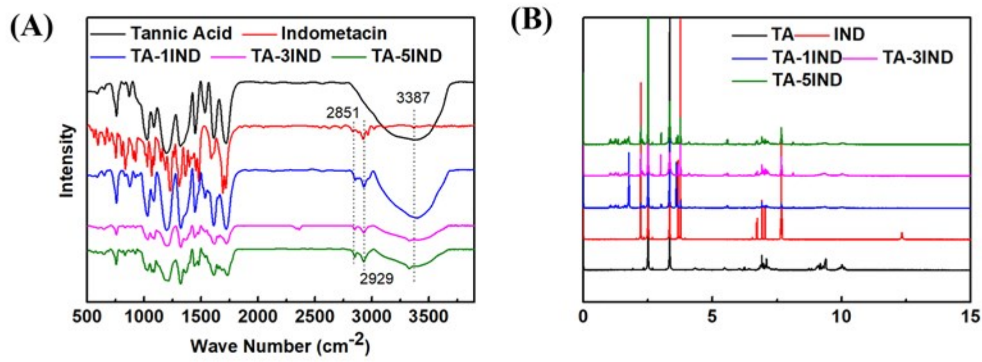

Figure S1. (A) FTIR spectrum of the synthesized TA-IND molecules; (B) ^{13}C NMR spectrum of the synthesized TA-IND molecules.

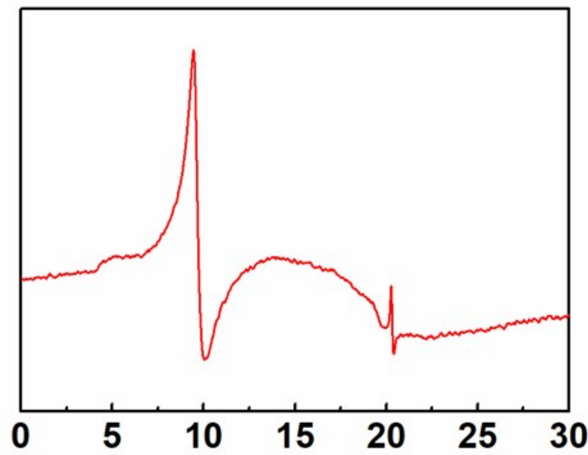

Figure S2. EPR spectra of the TA-IND/Fe^{3+} powder.

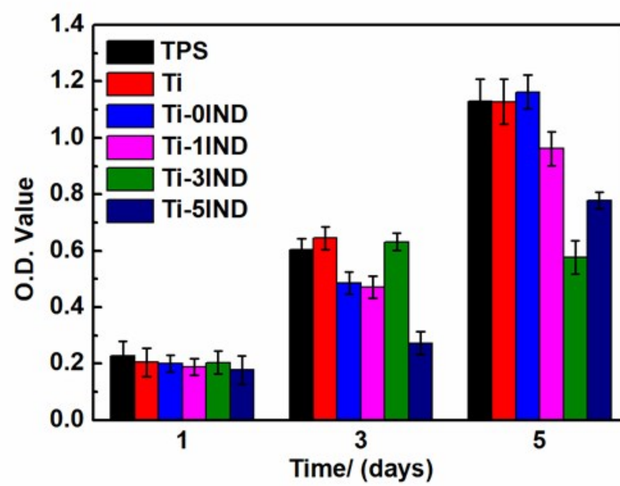

Figure S3. Proliferation profiles of HUVECs on the coatings.

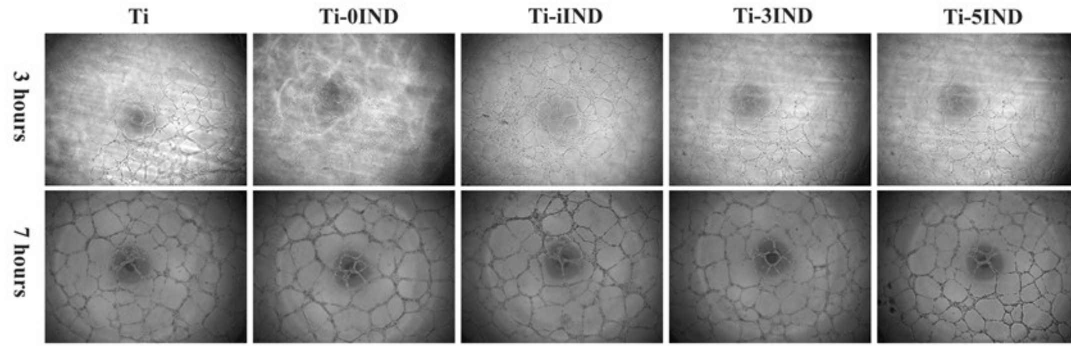

Figure S4. In vitro time-lapse microscopy images of the capillary network formed by HUVECs on ECMatrix gel with the specimen-conditioned medium.

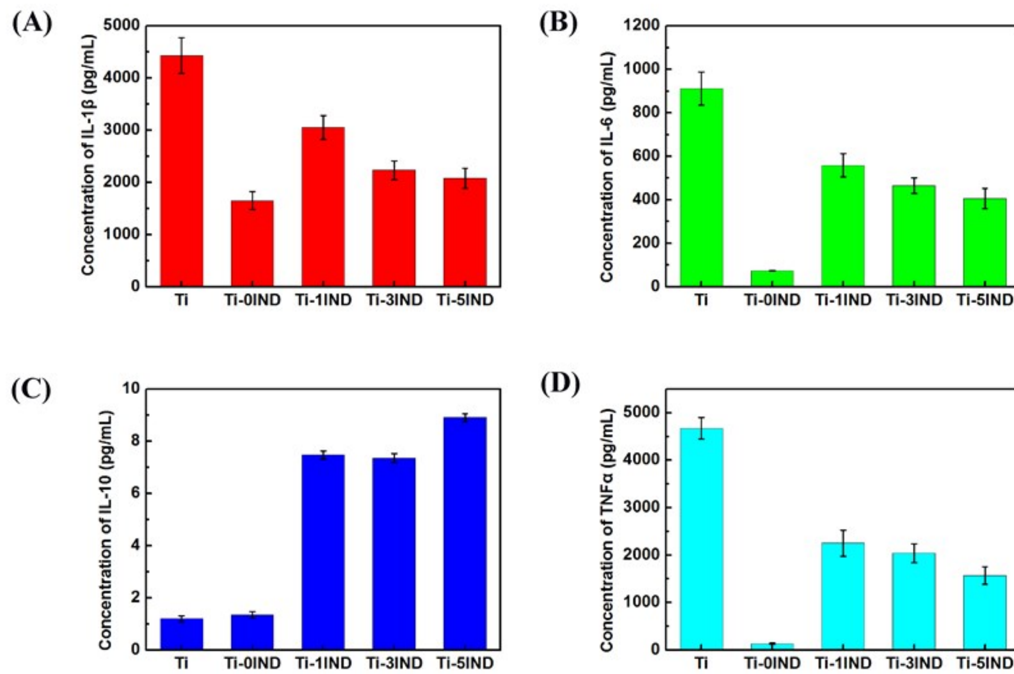

Figure S5. The concentrations of inflammatory-related cytokines secreted by the LPS treated macrophages seeded on the coatings.

Table S1. RNA Primers applied in this study

GENE	Forward primer (5′–3′)	Reverse primer (5′–3′)
GAPDH	TGACCACAGTCCATGCCATC	GACGGACACATTGGGGGTAG
IL-1β	TGGAGAGTGTGGATCCCAAG	GGTGCTGATGTACCAGTTGG
IL-6	ATAGTCCTTCCTACCCCAATTTCC	GATGAATTGGATGGTCTTGGTCC

IL-10	GAGAAGCATGGCCCAGAAATC	GAGAAATCGATGACAGCGCC
TNF-α	CTGAACTTCGGGGTGATCGG	GGCTTGTCACTCGAATTTTGAGA
INOS	CACCAAGCTGAACTTGAGCG	CGTGGCTTTGGGCTCCTC
CD206	AGACGAAATCCCTGCTACTG	CACCCATTCGAAGGCATTC
CD163	CGTGTGCAGTGTCCAAAAGG	CACAAACCAAGAGTGCCGTG
CD86	CTGCTCATCATTGTATGTCAC	ACTGCCTTCACTCTGCATTTG
BMP2	GCTCCACAAACGAGAAAAGC	AGCAAGGGGAAAAGGACACT
TGF-β1	CAGTACAGCAAGGTCCTTGC	ACGTAGTAGACGATGGGCAG
VEGF	GTCCCATGAAGTGATCAAGTTC	TCTGCATGGTGATGTTGCTCTCTG
ALP	AGGGTGGGTAGTCATTTGCATAG	GAGGCATACGCCATCACATG
OCN	CCGGGAGCAGTGTGAGCTTA	AGGCGGTCTTCAAGCCATACT
OST	CCCTTCTCAAGCACCAATGG	AGGGTGGGTAGTCATTTGCATAG
COL-1	CTCCGGCTCCTGCTCCTCTTA	ACCAGGAAGTCCAGGCTGTC