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Supporting Information

Construction of Three-dimensional Net-like Polyelectrolyte Multilayered Nanostructure onto Titanium Substrates for Combined Antibacterial and Antioxidant Application

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Table S1. Primers used for qRT-PCR in this study

Genes	Accession no.	Primers	Product size (bp)
Integrin β3	NM_153720.1	5’- TGACCCGCTTCAATGACGAA-3’	217
		5’- ATGGGTCTTGGCATCAGTGG-3’	
Integrin β1	NM_017022.2	5'-ACAAGAGTGCCGTGACAACT-3'	242
		5'-GTCTTCTGTCAGTCCCTGGC-3'	
Integrin α2	XM_001075558.5	5'-AGGGTACCATTCGCACCAAG-3'	163
		5'-AGTTGAACCACTTGCCCCAA-3'	
Integrin αv	NM_001106549.1	5’-TTATGCCAAAGATGACCCACT-3’	167
		5’- CGGGACCTCCAAGAAGTACTC-3’	
Col 1	NM_053304.1	5'-CCTGAGCCAGCAGATTGA-3'	106
		5'-TCCGCTCTTCCAGTCAG-3'	
ALP	NM_013059	5’-AGCGACACGGACAAGAAGC-3’	183
		5’-GGCAAAGA CCGCCACATC-3’	
Runx2	NM_053470.2	5’-GCCGTAGAGAGCAGGGAAGAC-3’	318
		5’-CTGGCTTGGATTAGGGAGTCAC-3’	
OCN	NM_013414.1	5’-GAGGGCAGTAAGGTGGTGAA-3’	154
		5’-CGTCCTGGAAGCCAATG TG-3’	
OPG	NM_012870.2	5’-ATTGGCTGAGTGTTCTGGTGG-3’	431
		5’-GACGGTTTTGGGAAAGTGGTAT-3’	
OPN	NM_012881.2	5’-GACAGCAACGGGAAGACC-3’	216
		5’-CAGGCTGGCTTTGGAACT-3’	
GAPDH	NM_017008.4	5’-GTTCCAATATGATTCCACCC-3’	400
		5’-TGAGTCCTTCCACGATACC-3’	

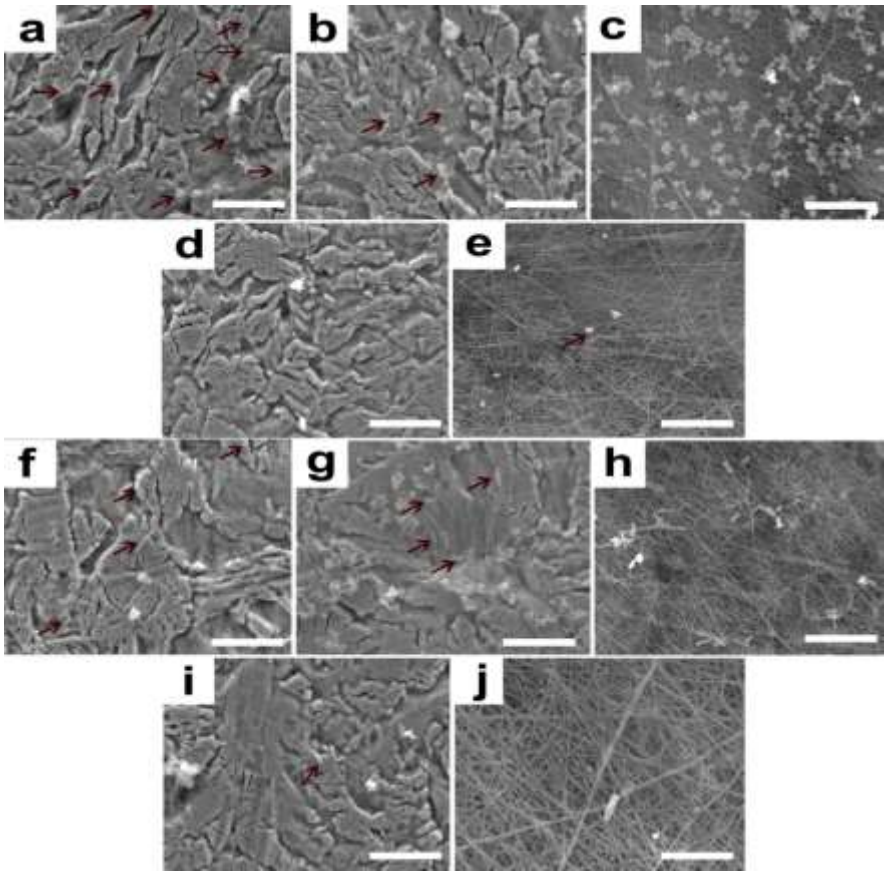

Figure S1.*In vitro* antibacterial treatment against *Staphylococcus aureus* on different Ti surfaces representative SEM images of *Staphylococcus aureus* adhered to: (a) Ti, (b) Ti-PDA, (c) Ti-PDA/NF, (d) Ti/LBL, (e) Ti-PDA/NF/LBL. Scale bar: 10μm;representative SEM images of *Escherichia coli* adhered to: (f) Ti, (g) Ti-PDA, (h) Ti-PDA/NF, (i) Ti/LBL, (j) Ti-PDA/NF/LBL. Scale bar: 10μm

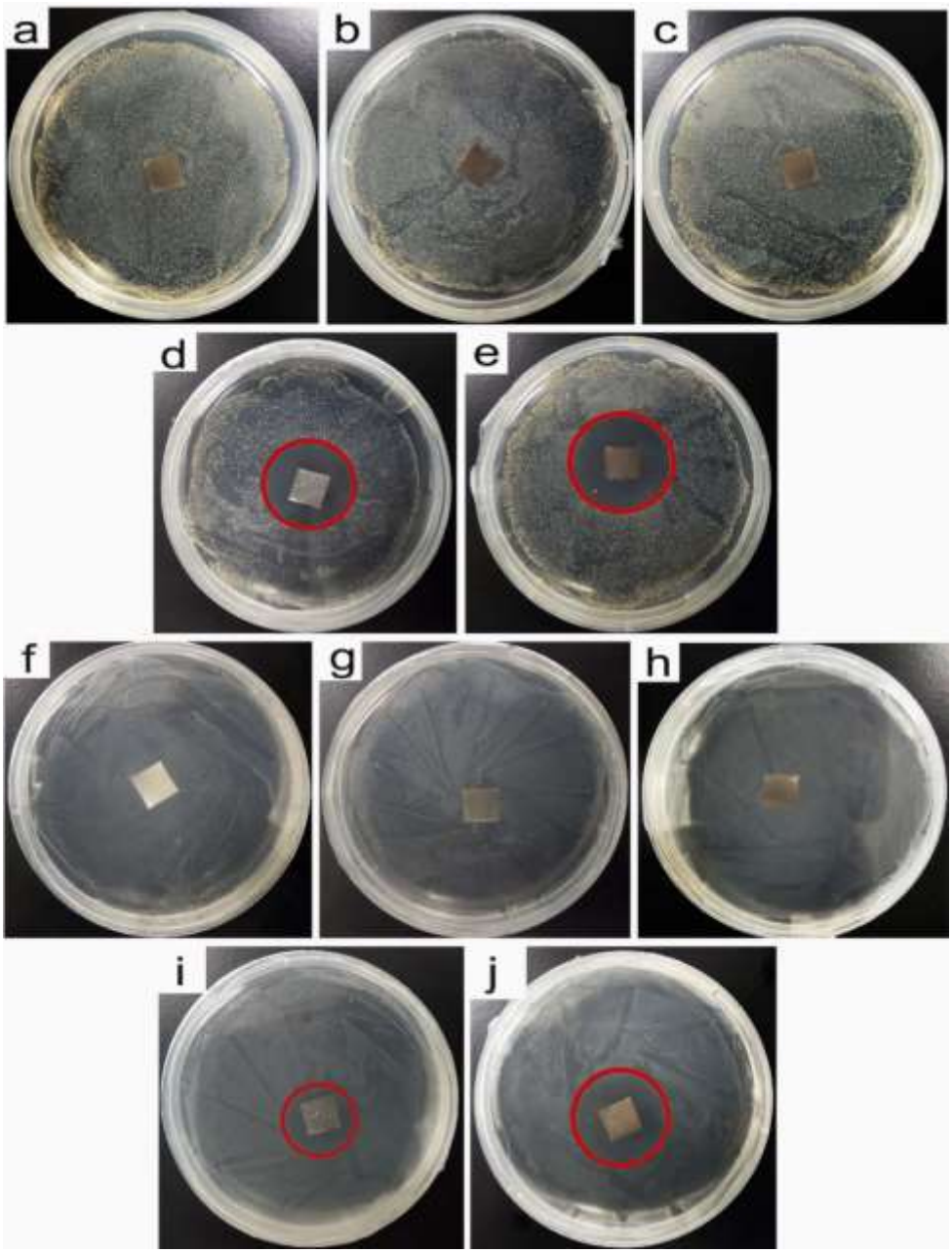

Figure S2. Images of Kirby-Bauer plates. Antibacterial properties against : *Staphylococcus aureus* of :(a) Ti, (b) Ti-PDA, (c) Ti-PDA/NF, (d) Ti/LBL, (e) Ti-PDA/NF/LBL; and *Escherichia coli* of: (f) Ti, (g) Ti-PDA, (h) Ti/LBL, (i) Ti-PDA/NF; (j) Ti-PDA/NF/LBL.

Protein adsorption on substrates

Bovine serum album (BSA) was used to evaluate protein adsorption of different substrates in this study. First, all of samples were immersed into BSA solution (1 mg/mL in PBS) at 37ºC for 4 h. After washing with PBS, SDS was used to extract the protein absorbed by the substrates. The amount of extracted protein was quantified with BCA assay kit using spectrophotometric plate reader at 370 nm.

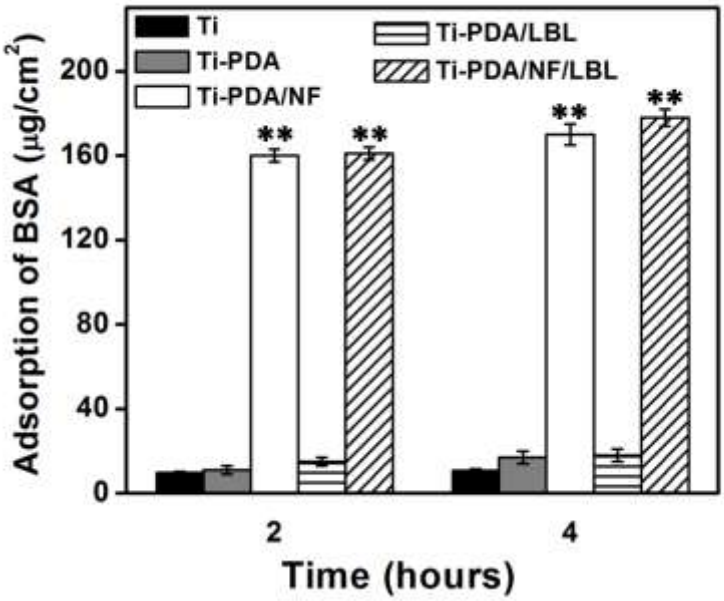

Fig. S3. Protein adsorption assay of different substrates. Ti was used as control for the statistical analysis. Error bars represent mean ± SEM for n=4, **p<0.01

Protein adsorption is an important factor closely related to the cytocompatibility of implant material, since the surface adsorbed proteins would further affect a series of cellular behaviors (e.g., cell adhesion, spreading, proliferation, differentiation, etc.). As shown in Fig S3, Ti-PDA/LBL showed higher protein adsorption compared to native Ti substrates. BSA could precipitate with TA. Therefore, the introduction of TA would result in higher protein adsorption. The result also showed that Ti-PDA/NF and Ti-PDA/NF/LBL showed highest protein adsorption among all groups. This may be attributed to the higher level of chitosan in these two substrates. At physiological pH, the protonated amino groups in chitosan would interact with the negatively charged BSA, leading to the enhanced adsorption of albumin and improving its affinity with BSA at the surface. Notably, the amount of absorbed protein was highest for Ti-PDA/NF/LBL, which was the combined result of BSA-chitosan / BSA-TA interaction and larger surface area. The observation was also consistent with several previous studies.[1, 2]

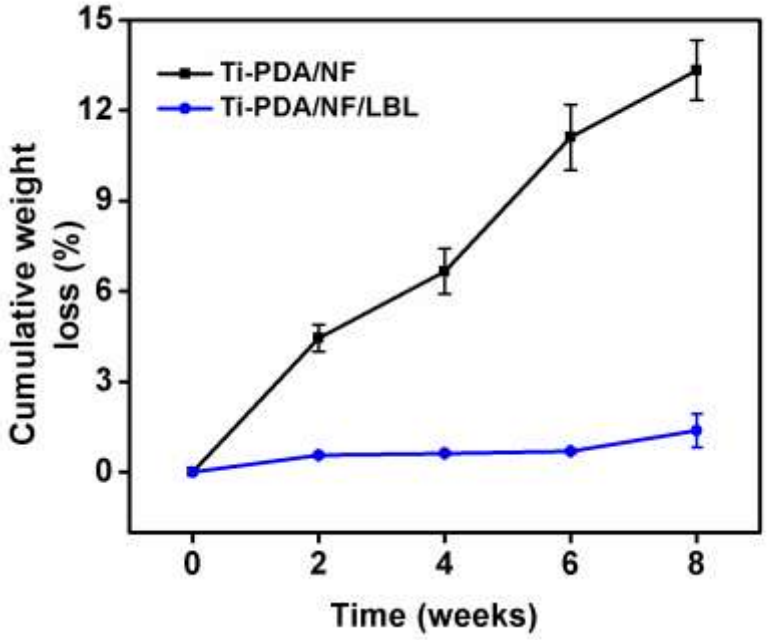

Fig. S4 *In vitro* degradation profile of Ti-PDA/NF.and Ti-PDA/NF/LBL

Additional References

1. Y. Gao, Y. B. Wang, Y. M. Wang and W. G. Cui, *Mar. Drugs*, 2016, **14**.
2. C. Wu, W. Y. Chang, H. Z. Qi, L. X. Long, J. Zhao, X. B. Yuan, Z. Y. Li and X. J. Yang, *J. Coat. Technol. Res.*, 2017, **14**, 1127-1135.