Supporting Information

A bio-inspired, one step and versatile coating onto various substrates with strong antibacterial and enhanced osteogenesis

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Experimental Section

Materials

Poly(lactic acid), poly(styrene), and poly(propylene) were purchased from Aladdin. Tannic acid (TA), Polyethylenimine (PEI, M_w ~ 10 k), Silver nitrate, Gold(III) chloride hydrate, $NH_3 \cdot H_2O$, H_2SO_4, phosphophate buffer, and 4',6-diamidino-2-phenylindole (DAPI) were purchased from Sigma. 4% paraformaldehyde, phalloidin-TRITC, Triton X-100, were purchased from Solarbio Life Science. MEN-α medium and FBS were purchased from Gibco. TRIzol reagent was purchased from Sangon Biotech. SYBR Green PCR Kit and PrimeScript RT reagent kit were purchased from TAKARA. Cell Counting Kit-8（CCK-8）was purchased from Dojindo Laboratories. All chemicals were used without any further purification. Silicon wafers (SSP), quartz plates (Alfa Aessar), round glass coverslips (14 mm diameter from NEST) were cleaned by piranha solution (70% H_2SO_4 and 30% H_2O_2, V/V) at 80 °C for 1h, followed by rinsing with Milli-Q water and subsequently dried under a mild stream of purified nitrogen gas before use. Deionized water used here was purified through a Milli-Q system with a resistivity greater than 18 MΩ·cm.

Preparation of TA/Ag or TA/Au coatings

For TA/Ag coating, TA solution (10mg/mL, 4mL) were directly added to mixed solution of PEI（1mg/mL, 400uL) and Ag^+ (1M, 800uL) with substrates on vortex at room temperature in just few minutes (10s, 30s, 60s, 300s, 600s), and these samples were noted as TA/Ag-time. The 300s procedure can be repeated, and the obtained samples are noted as TA/Ag-300s-R3, R3 was noted as the repeating number. For

TA/Au coating, the concentration of $AuCl_4^-$ was 0.1M with the same process of preparing TA/Ag coating. All samples were rinsed 3 times with Milli-Q water and subsequently dried under a mild stream of purified nitrogen gas.

Cell isolation and proliferation

Human dental pulp mesenchymal stem cells (DPSCs) were isolated from the pulp of human exfoliated deciduous teeth. The growth medium was prepared by addition of 100 U/ml penicillin, 100 mg/ml streptomycin and 10% FBS into MEN-α medium. The cells were cultured in a 5% CO_2 incubator at 37 °C. Non-adherent cells were removed after 2 days, and then fresh growth media were added. The stem cells were passaged upon almost confluence.

Stem cell seeding

After sterilization, the glasses (14×14 mm) with TA/Ag modified surface was transferred into 24 well plates, and then DPSCs were seeded in each well with a normal density 5000. After 2 h of incubation, the non-adherent cells were removed, and the fresh growth medium was added into the wells. After cell culture on the glasses for 7 and 14 days, the cell counting kit-8 was used to quantify the total cell viability according to the manufacturer's instruction. The total viability indirectly reflects the relative number of adherent cells.

Immunocytochemistry observation

Stem cells on glasses were rinsed carefully with phosphate buffer saline (PBS) solution after 7 days and 14 days of incubation in the growth medium. Then 4% paraformaldehyde was used to fix cells. After washing the samples with PBS three

times (5 min per wash), the cells were permeabilized with 0.1% Triton X-100 in PBS for 5 min, and labeled the microfilaments by phalloidin-TRITC (1 mg/ml) for 15 min. At last, cells were treated by DAPI (2 mg/ml) for 5 min to stain cell nuclei, and then all the stained samples were rinsed with deionized water thoroughly. Cells were observed on an inverted fluorescence microscope.

Real-time polymerase chain reaction (RT-PCR) analysis

The expression levels of the osteogenic-related genes alkaline phosphatase (ALP), Osteonectin (ON), osteocalcin (OCN) and osteopontin (OPN) were measured using qRT-PCR. The toTAl RNAs of stem cells on glasses （control groups） and TA/Ag coatings after 7 days or 14 days in the co-induction medium were extracted using the TRIzol reagent. The 1st strand of complementary DNA (cDNA) was reversely transcribed from RNA, using PrimeScript RT reagent kit according to the manufacturer's instructions. Quantitative PCR was set up in a total volume 20 μl with corresponding primers (shown in Table S1) and SYBR Green PCR Kit. The results were normalized by the mean values of the corresponding control groups.

Antibacterial test

Bacteria in the mid-exponential growth phase were harvested by centrifugation and resuspension in PBS buffer solutions (pH = 7.2) for *S. aureus* (ATCC #6538) and *E. coli* (ATCC #8739）) to a final bacteria number of 1×10^8 in 10 mL solution. Add 1 mL bacteria solution to each well of 24-well plates containing silicon wafer or TA/Ag coatings at the bottom. The suspensions were incubated for additional 6 h at 37°C, washed three times with PBS, and prefixed with 4% paraformaldehyde for 15

min. The substrates were subsequently washed three times with PBS and dried under vacuum for 12 h for FSEM measurements. The morphological change of the bacterial cells on TA/Ag coatings was examined using FSEM. At least 100 bacteria or bacteria on 10 random images (with total area ~ 4×10^3 um^2) were counted in statistical analysis.

Charateriazation

UV-vis spectra of the multilayers deposited on quartz slides were collected on a UV-vis Spectrometer (Lambda 25, PerkinElmer). Contact angle analyses were carried out using the static sessile drop method on a KRUSS DSA1 version 1.80 drop shape analyzer with water as the probe liquid. Each contact angle value reported was an average of at least five measurements. X-ray photoelectron spectra (XPS) were obtained on a Thermo-Electron ESCALAB 250 spectrometer equipped with a monochromatic Al X-ray source (1486.6 eV). The spectrawere recorded at 90 takeoff angle with 20 eV pass energy. The high takeoff angle was used in order to assess the bulk film composition instead of the film surface only, which was confirmed by the Si peaks observed for the wafer substrate. The thickness of TA/Ag coating was determined by optical ellipsometry (EX2, ELLITOP). The measurement of each sample was repeated three times, and the average values were reported. The surface morphology of TA/Ag coatings were characterized by FSEM (SU8010, HITACHI) in SE (L) mode. The electron beam was operated at 3,000 V and 10 μA, without the use of additional conductive coating. The elemental composition mappings of C, N, Ag were measured for 30min by EDS analysis of FSEM at 15000 V. Inverted

fluorescence microscope (DMi8, Leica) and qRT-PCR(LightCycler 96, Roche) were used. The RNA purity was checked by NanoDrop (DeNovix). Vortex(MX-S,SCILOGEX) was used. All quantitative data were statistically analyzed using t test. $P < 0.05$ was considered statistically significant, noted as "*". For $P < 0.01$ noted as "***", no significant noted as "NS". Results were presented as mean standard deviation.

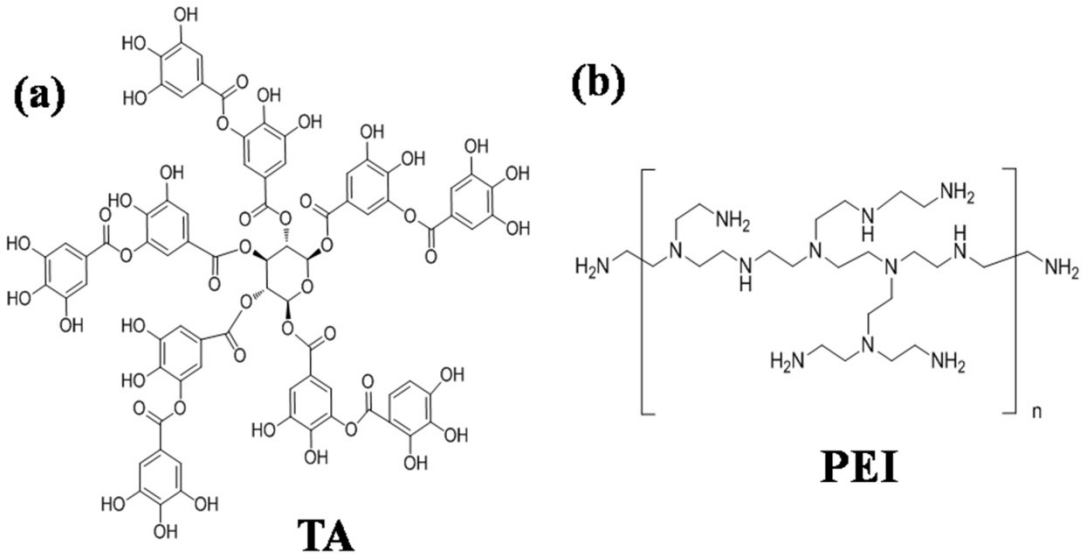

Figure S1. The chemical structural formula of Tannic acid (TA) and polyethylenimine (PEI)

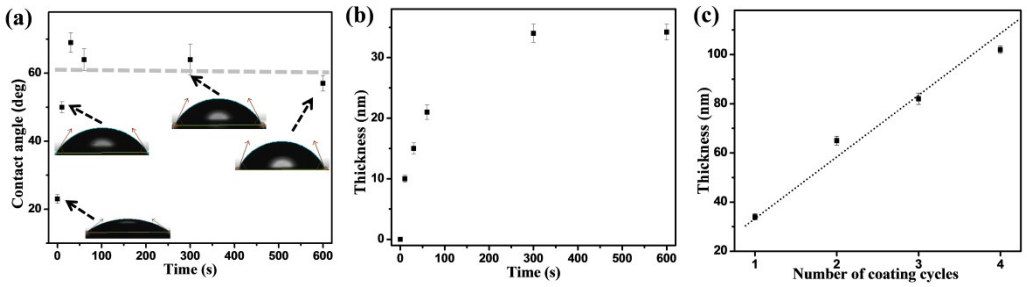

Figure S2. (a) Water contact angle of TA/Ag coatings on deposition time. (b) Coated thickness on deposition time. (c) Dependence coated thickness on number of coating cycles.

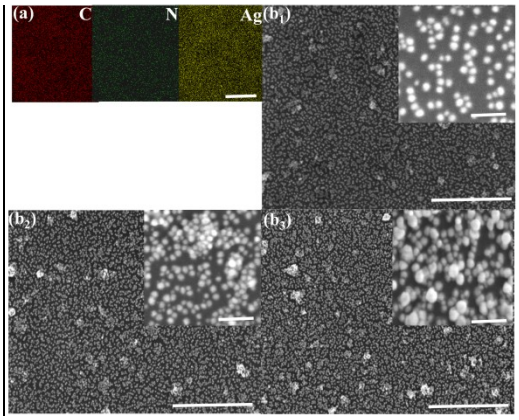

Figure S3. (a) EDS elemental mapping of TA/Ag coating. (b) SEM images of TA/Ag coatings on silicon with vortex time 10s (b_1), 60s (b_2) and 300s (b_3). The scale bar is 200 nm in (a), 10 um in (b), 100 nm in the insets of (b).

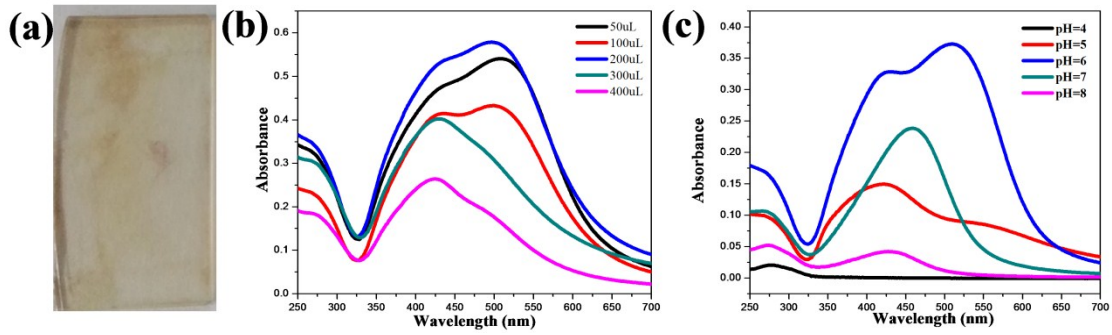

Figure S4.（a）The photo of TA/Ag coatings on quartz without PEI.（b）The UV-vis spectra of TA/Ag coatings with different amount of PEI.（c）The UV-vis spectra of TA/Ag coatings with different pH.

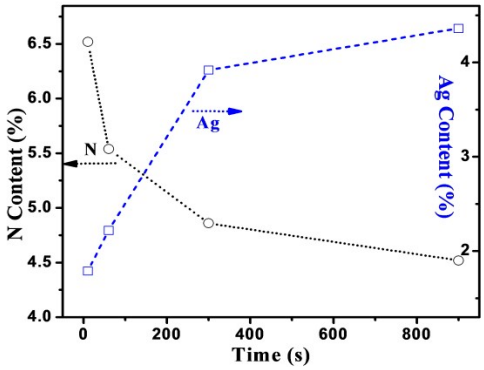

Figure S5. Dependence of nitrogen and silver content and on deposition time.

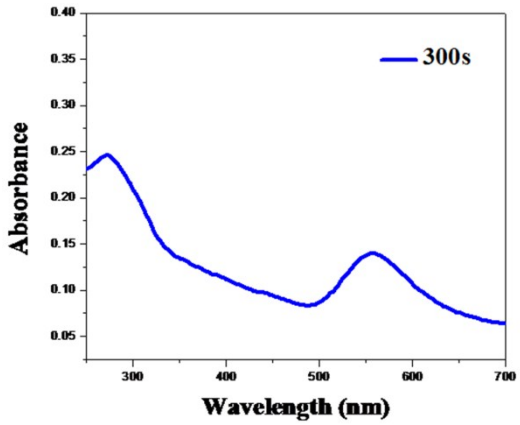

Figure S6. The UV-vis spectra of TA/Au coatings on quartz

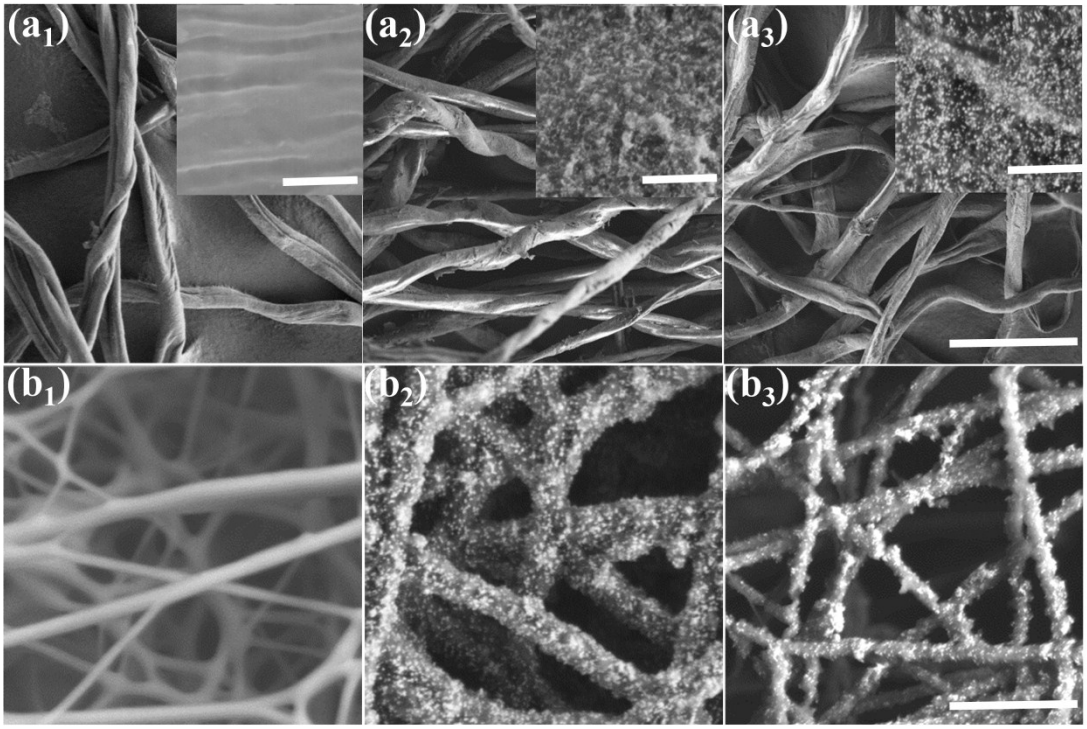

Figure S7. SEM images of original cotton (a_1) and PLA fibers (b_1), after coating with TA/Ag^0 (a_2, b_2) and TA-gold (a_3, b_3). The insets are the magnified images of corresponding samples. The scale bar is 100 um in a, 1um in b, 500 nm in the insets of a.

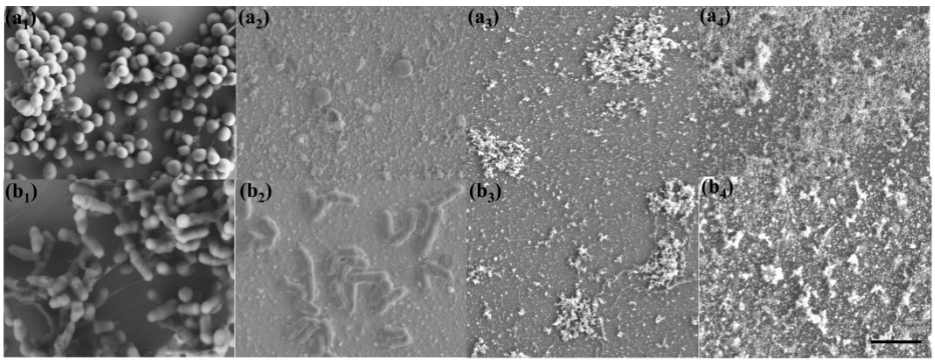

Figure S8. SEM images of (a) S. aureus and (b) E. coli after exposure to silica wafer (1 as control), TA/Ag coatings fabricated with different deposition time(2, 3, 4 refer to 60s 300s and 300s-R3 respectively). The scale bar is 5 um.

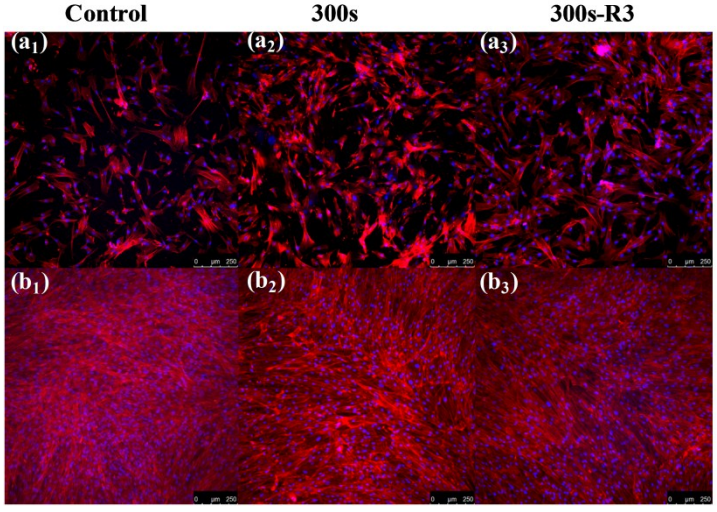

Figure S9. Fluorescent images of actin cytoskeleton of DPSCs stained with rhodamine phalloidin at day 7 (a) and 14 (b).

Table. S1 Compiled list of gene targets probed in this study.

ON	forward TCGGCATCAAGCAGAAGGATA
	reverse CCAGGCAGAACAACAAACCAT
OCN	forward GTGACGAGTTGGCTGACC
	reverse TGGAGAGGAGCAGAACTGG
OPN	forward CTCCATTGACTCGAACGACTC
	reverse CAGGTCTGCGAAACTTCTTAGAT
ALP	forward GTATCGGCAGCAGTCAGCAGTG
	reverse TCCAGGCAGGCGGCGAAG

Table S2. Composition of coatings of N_{1s} at 399.5 ev and 401.7 ev tested by XPS.

Samples	PEI	TA/Ag-10s	TA/Ag-60s	TA/Ag-300s	TA/Ag-300s-R3
Composition of N_{1s} at 399.5 eV	100	72.27	62.06	58.67	53.72
Composition of N_{1s} at 401.7 eV	0	27.73	37.94	41.33	46.28