Electronic Supplementary Information (ESI)

Composited Silk Fibroins Ensured Adhesion Stability and Magnetic Controllability of Fe_3O_4-Nanoparticles Coating on Implant for Biofilm Treatment

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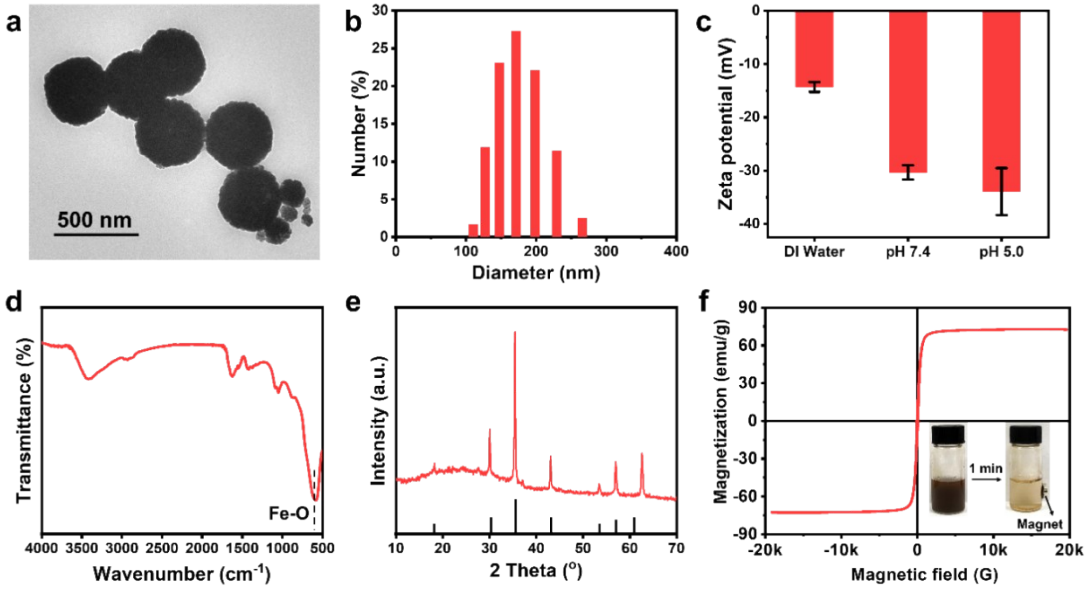

Fig. S1 Characterization of Fe_3O_4 nanoparticles.

(**a**) Transmission electron microscope micrograph of Fe_3O_4 nanoparticles.

(**b**) Diameter distribution of Fe_3O_4 nanoparticles suspended in deionized (DI) water.

(**c**) Zeta potentials of Fe_3O_4 nanoparticle suspended in DI water or in phosphate buffered saline (PBS, pH 7.4 and 5.0). Values are expressed as means ± standard deviations over three parallel groups with different batches of nanoparticles.

(**d**) Fourier transform infrared (FTIR) spectra and (**e**) X-ray diffraction (XRD) pattern of Fe_3O_4 nanoparticles.

(**f**) Magnetic hysteresis loops of Fe_3O_4 nanoparticles measured by vibrating sample magnetometry at 300 K. Inset in the lower right corner shows the magnetic behavior of Fe_3O_4 nanoparticles under an applied external magnetic-field.

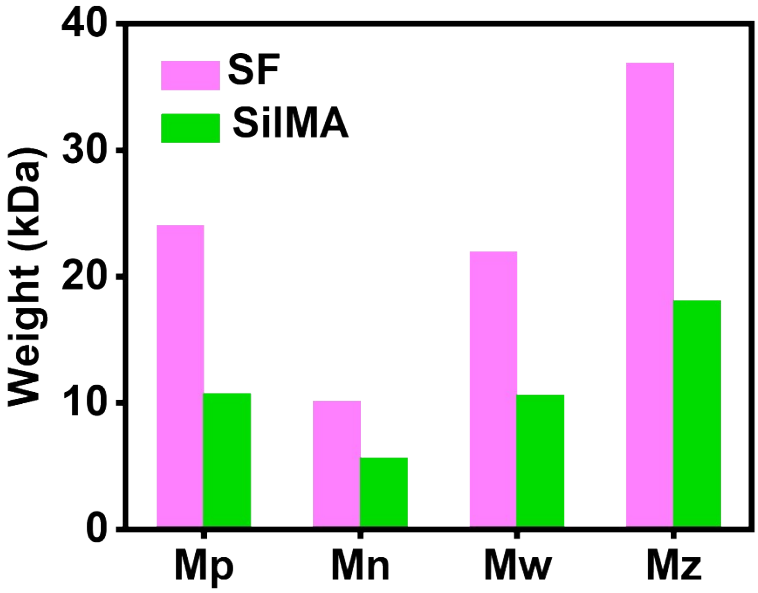

Fig. S2 Molecular weight of SF and SilMA polymers as measured by gel permeation chromatography. Mp for peak molecular weight, Mn for number-average molecular weight, Mw for weight-average molecular weight and Mz for average molecular weight.

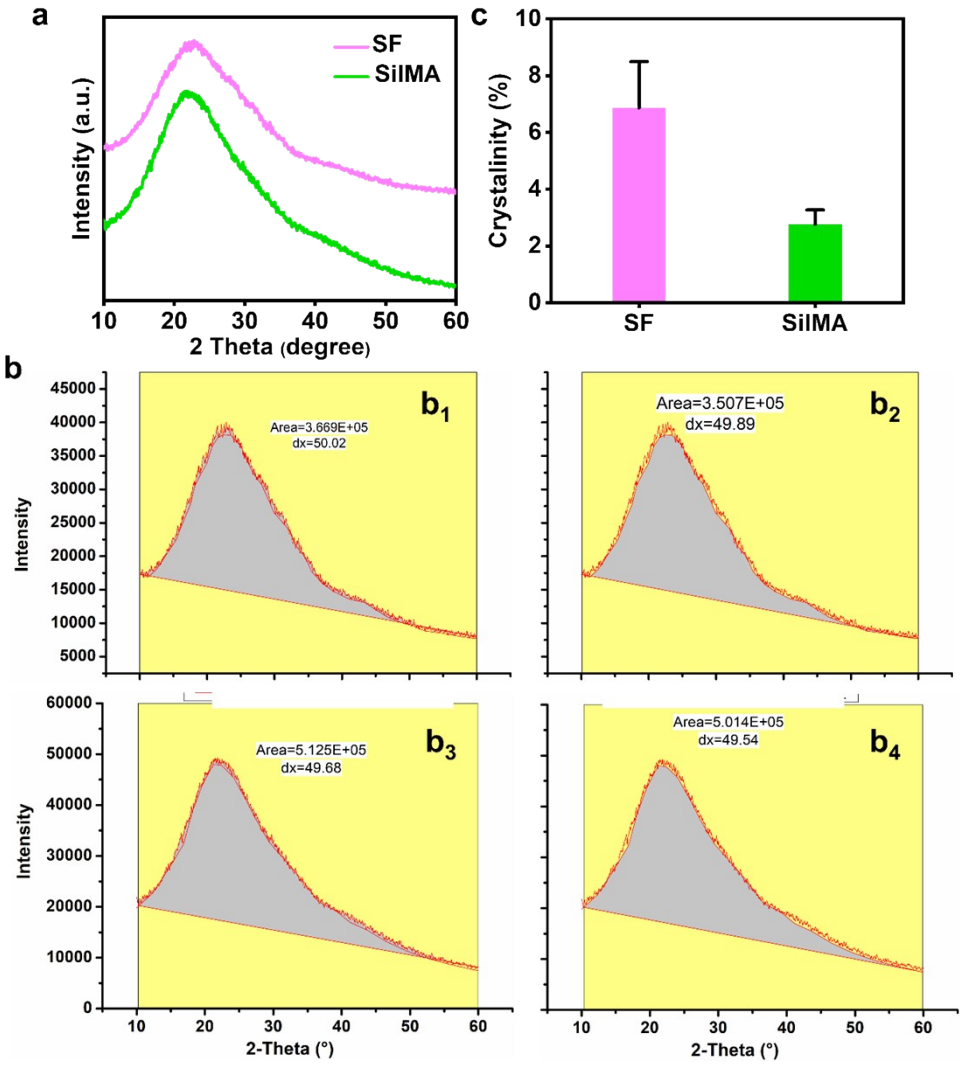

Fig. S3

(**a**) X-ray diffraction patterns of SF and SilMA coatings.

(**b**) Schematic diagram of calculating the total area D of crystallized and non-crystallized zones using origin, and calculating the area D1 of the non-crystallized zone; b1 and b2 are calculation diagrams of D and D1 of SF, respectively; b3 and b4 are calculation diagrams of D and D1 of SilMA, respectively.

(**c**) Crystallinity of SF and SilMA coating as calculated by XRD results from panle b). Crystallinity (%) = $(D - D_1) / D \times 100\%$.

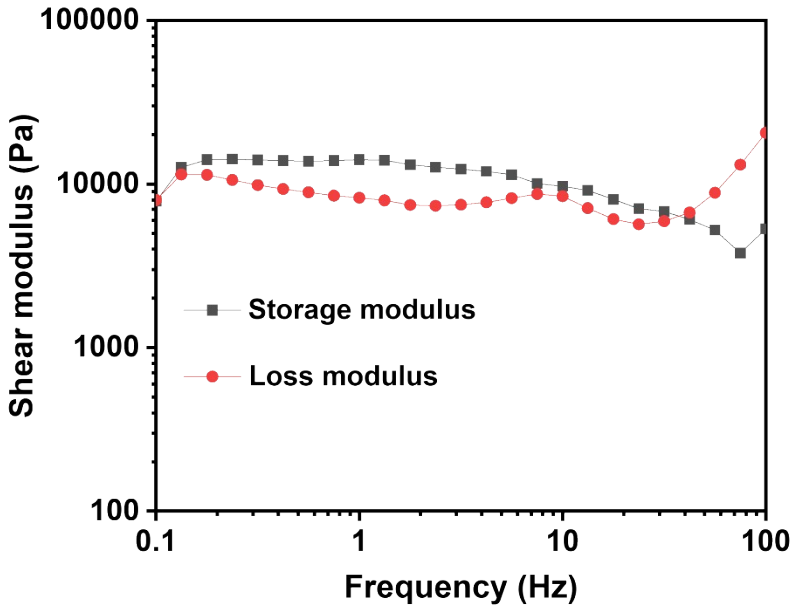

Fig. S4 Frequency sweep from dynamic angular shear tests in the frequency range of 0.1-100 Hz of Fe_3O_4/SFMA coatings on Ti.

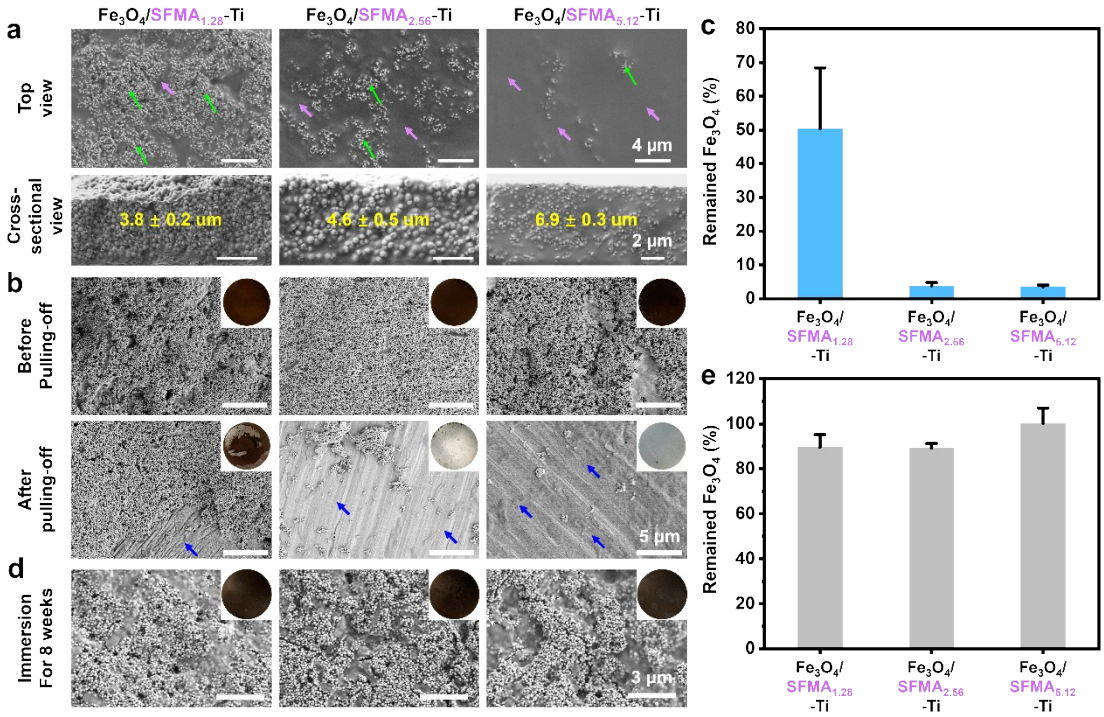

Fig. S5 Coating densities of SFMA on Ti surfaces.

(**a**) Top view and cross-sectional view of SEM micrographs of Fe_3O_4/SFMA coatings on Ti discs in different SFMA coating densities, ranging from 1.28 to 2.56 and 5.12 mg/cm^2. Green arrows point out the exposured Fe_3O_4 nanoparticles and pink arrows point out SFMA. The average thickness of each coating was measured from cross-sectional view.

(**b**) Macroscopical and microscopical observation of Fe_3O_4/SFMA coatings after magnetic pulling-off. Black color in real pictures indicates Fe_3O_4 nanoparticles. Blue arrows point out Ti surface and pink arrows point of SFMA in SEM micrographs. The diameter of the Ti discs in the real pictures is 10 mm.

(**c**) Quantification of Fe_3O_4 content that remained on Ti surfaces after magnetic pulling-off as measured using ICP-MS with respect to the content of Fe_3O_4 content on each surface prior to magnetic pulling-off from panel (c).

(**d**) SEM micrographs and real pictures of each coating on Ti after 8-weeks' immersion in PBS (pH 7.4) at 37^{o}C without magnetic pulling-off. The diameter of the Ti discs in the real pictures is 10 mm.

(**e**) The quantification of remaining Fe_3O_4 nanoparticles on Ti from panel (d) as measured using ICP-MS. 100% is the amount of Fe_3O_4 nanoparticles on each surface before immersion. All data are expressed as means ± standard deviations over three samples.

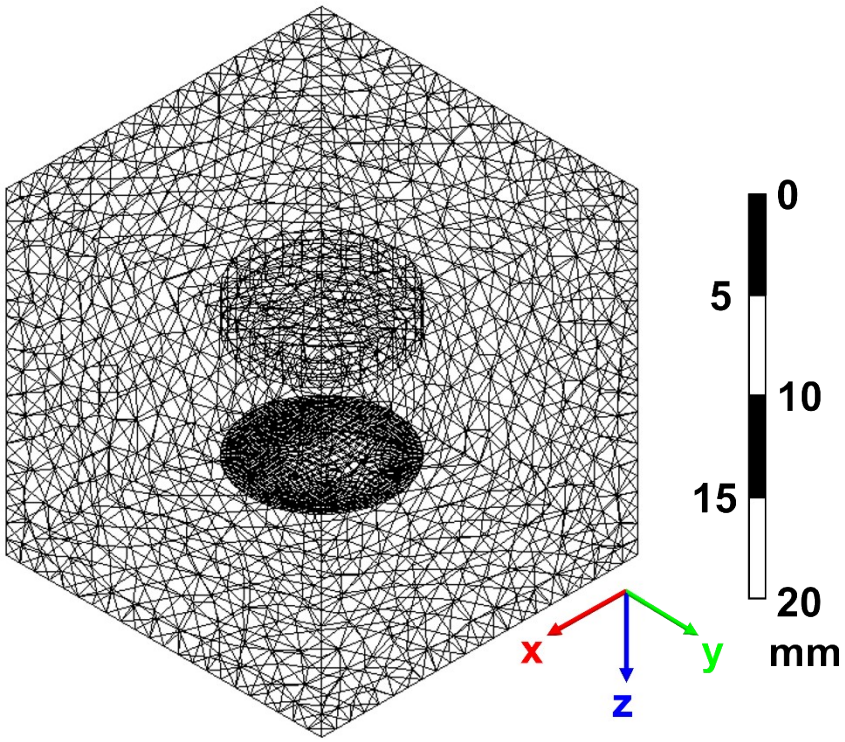

Fig. S6 Model of Magnetomechanic simulation with magnet on the top and Fe_3O_4/SFMA coatings at the bottom.

Table S1. Minimal inhibitory (MIC) and minimal bactericidal (MBC) concentrations of gentamicin, vancomycin and Fe_3O_4 nanoparticles for Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* (*E. coli*).

	MRSA		E. coli	
	MIC ($\mu g\ mL^{-1}$)	MBC ($\mu g\ mL^{-1}$)	MIC ($\mu g\ mL^{-1}$)	MBC ($\mu g\ mL^{-1}$)
Gentamicin	20.3 ± 7.2	50.0	1.0 ± 0.4	2.5
Vancomycin	1.0 ± 0.5	2.1 ± 0.9	166.7 ± 72.2	250.0
Fe_3O_4 nanoparticles	> 500		> 500	

Data are expressed as means ± standard deviation over three experiments with separately cultured bacteria.

Method: Bacterial strains were grown as described in the experimental section. 100 µL of gentamicin (100 $\mu g\ mL^{-1}$), vancomycin (1 $mg\ mL^{-1}$) or Fe_3O_4 nanoparticles (1 $mg\ mL^{-1}$) in PBS was added into a 96 wells-plate containing 100 µL of TSB per well. Solutions were 2-fold serially diluted, followed by the addition of 10 µL of bacterial suspension (10^5 CFU mL^{-1}) to each well. The suspension was incubated for 24 h at 37°C and the minimal inhibitory concentration (MIC) was taken as the lowest concentration at which no visual growth was observed. Then, 10 µL of the bacterial suspension was taken from each well showing no visual growth and dropped on an agar plate. After 24 h incubation at 37°C, the lowest concentration at which no colonies were formed on the agar plate was taken as the MBC value. The experiment was performed in triplicate with separately cultured bacteria.

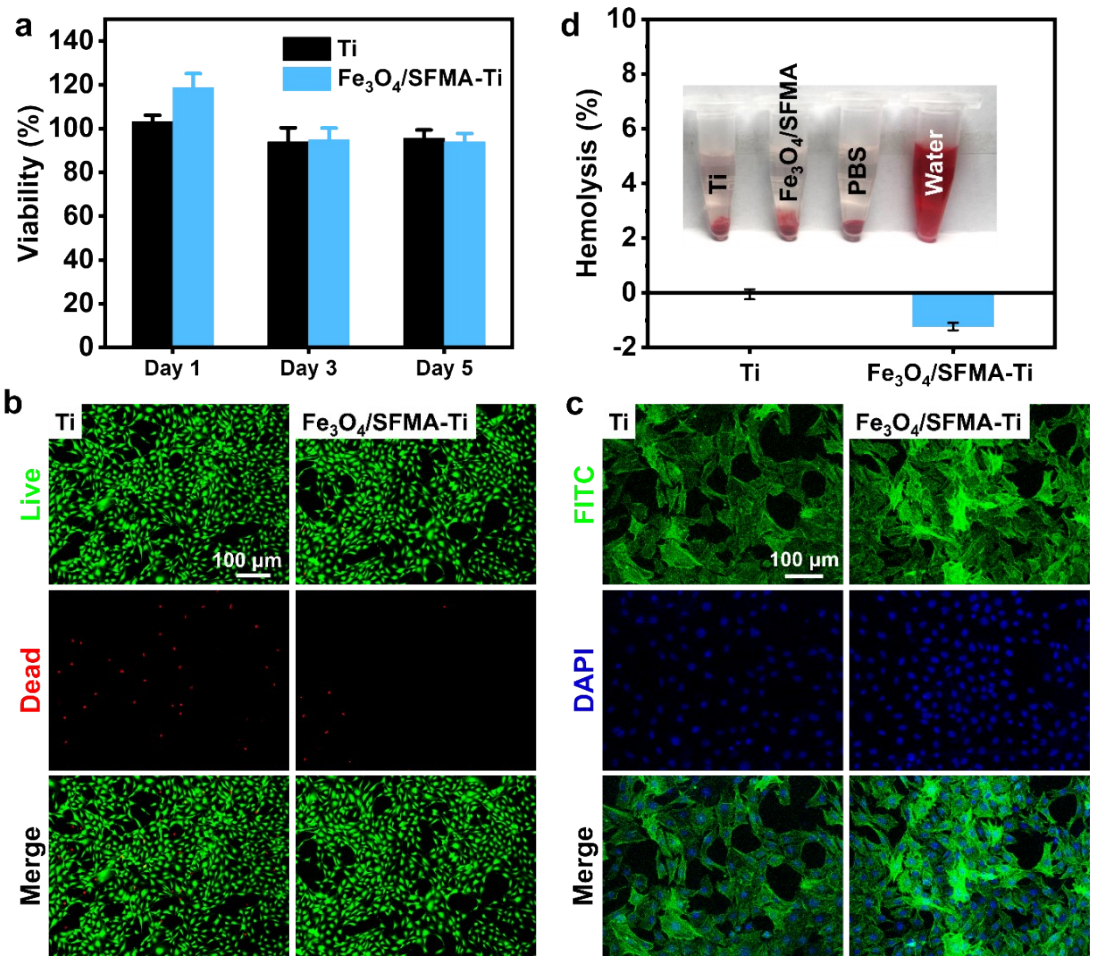

Fig. S7 Biocompatibility of Fe_3O_4/SFMA coating on Ti surface.

(**a**) CCK-8 tests of MC3T3-E1 cells cultured in extracts from each sample for 1, 3 and 5 days. Data are expressed as means ± standard deviations over five parallel samples.

(**b**) Fluorescent images of live/dead staining of MC3T3-E1 cells after culturing for 1 day in extracts.

(**c**) Fluorescent images of MC3T3-E1 cells cultured for 1 day with actin stained with FITC in green color and nuclei stained with DAPI in blue color.

(**d**) Relative hemolysis of mouse red blood cells after 3 h exposure at 37°C to magnetic nanoparticles. Relative hemolysis was derived from UV absorption at 540 nm, setting hemoglobin absorption of cells exposed to ultrapure water at 100%. Data are expressed as means ± standard deviations over three parallel samples. Inserted graph showed RBCs suspensions of each group after centrifugation.

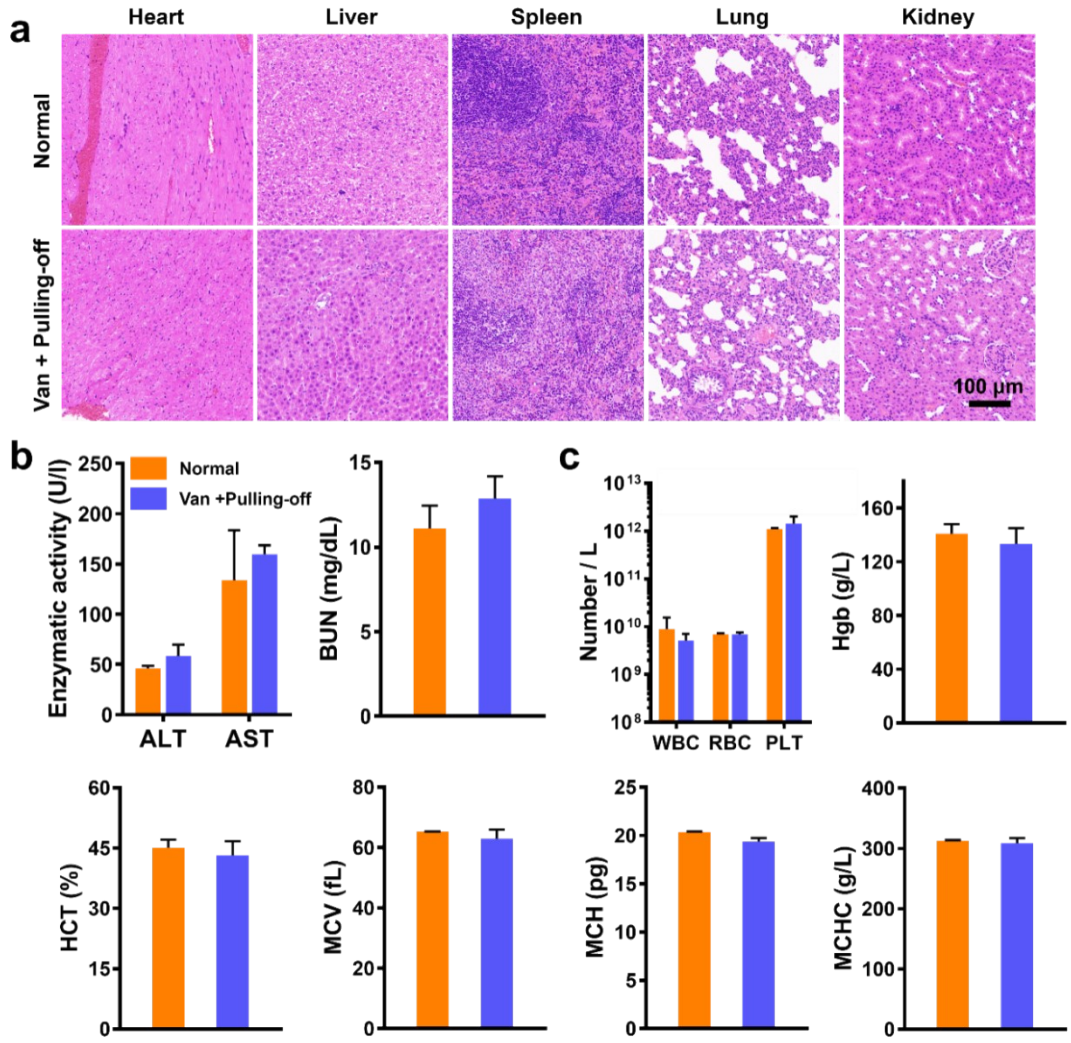

Fig. S8 *In vivo* biosafety evaluation of IAIs treatments on Fe_3O_4/SFMA surface.

(a) H&E staining for histological analysis of excised tissues, including heart, liver, spleen, lung and kidney from normal rats and IAIs bore rats after vancomycin administration and magnetic pulling-off Fe_3O_4 nanoparticles. Normal rats were raised in the same condition with experimental rats but without implantations and treatments. All rats were sacrificed at day 7.

(b) Blood chemistry and (c) routine blood parameters of the rats at day 7. ALT stands for alanine transferase, AST for aspartate transferase, BUN for blood urea nitrogen, WBC and RBC for white and red blood cell respectively, PLT for platelets, Hgb for hemoglobin, HCT for hematocrit, MCV for mean cell volume, MCHC for mean corpuscular hemoglobin concentration and MCH for mean corpuscular hemoglobin. Data are expressed as means ± standard deviations over three parallel samples from three rats.

MATERIALS AND METHODS

Materials. Iron (III) chloride ($FeCl_3 \cdot 6H_2O$), ethylene glycol $(CH_2OH)_2$, sodium acetate (CH_3COONa) were purchased from Sinopharm Chemical Reagent Co. (China). Sodium carbonate (Na_2CO_3), lithium bromide (LiBr), poly (ethylene glycol) (MW 10,000), sodium chloride (NaCl), monopotassium phosphate (KH_2PO_4), dipotassium phosphate (K_2HPO_4) and ethanol were purchased from Aladdin (China). Silk fibroin methacryloyl (SilMA) was purchased from Suzhou Yongqinquan Intelligent Equipment Co., Ltd. (China). All chemicals were used as received.

Synthesis of Fe_3O_4 nanoparticles. Fe_3O_4 nanoparticles were synthesized according to a previously described solvothermal method.[1] Briefly, 1.35 g of $FeCl_3 \cdot 6H_2O$ was dissolved in 40 mL of ethylene glycol and then 7.2 g of sodium acetate was added. After magnetically stirring for 60 min, the mixture was transferred into a Teflon-lined stainless-steel autoclave and reacted at 200°C for 6 h. After cooling down to room temperature (RT), the black nanoparticles were magnetically separated and washed with distilled water and ethanol each for 3 times and then dried at 60 °C overnight for later use.

Preparation of Silk Fibroin. Silk fibroin (SF) was prepared according to a previous described method.[2] Briefly, silkworm cocoons were boiled in Na_2CO_3 (0.02 M) solution for 30 min and then dissolved in LiBr solution (9.3 M) at 60°C. The mixture was then dialyzed in distilled water in a dialysis bag (12,000-14,000 MWCO, MEMBRA-CEL, Germany) under mildly stirring for 72 h at RT with refreshing the distilled water every 12 h. Then the mixture was centrifuged at 8000 rpm for 20 min to remove the impurities, yielding a ~20% (w/v) SF solution. The SF solution was further concentrated by dialyzed reversely in a PEG (MW 10,000) solution (150 mg/mL) into a concentration of 50% (w/v) and kept at 4°C for later use.

Preparation of Fe_3O_4/SFMA on Ti. Fe_3O_4 nanoparticles were firstly dispersed in distilled water under sonication and then mixed with SF (50% w/v) and SilMA (50% w/v) by vortex. The mixture was dropped on a Ti disc (10 mm in diameter and 1 mm in thickness, polished with 800 and 2000 mesh sandpapers, respectively), irradiated under ultraviolet (UV) light for 30 seconds and then dried at 37°C. The surface

concentration of Fe_3O_4 nanoparticles on Ti disc was 1.28 mg/cm^2 to fully cover the surface according to our previous work.[3] The surface concentration of SFMA was 2.56 mg/cm^2 to fully trap Fe_3O_4 nanoparticles according to Scanning Electron Microscopy (SEM; S-4700, Hitachi, Japan) observations which were not shown here.

Characterizations of Fe_3O_4 nanoparticles, SFMA and Fe_3O_4/SFMA-Ti. Transmission electron microscopy (TEM, G-120, Hitachi, Japan) was used to observe the size and shape of Fe_3O_4 nanoparticles. To this end, nanoparticles suspended in ethanol were dropped on a carbon coated copper grid and dried at RT before imaging. Hydrodynamic diameter was measured by dynamic light scattering (DLS, Malvern ZetaSizer ZS2000, UK) in phosphate buffered saline (PBS, 5 mM K_2HPO_4, 5 mM KH_2PO_4, 150 mM NaCl, pH 7.4). Zeta potential was measured by suspending Fe_3O_4 nanoparticles in PBS (pH 7.4) using a Malvern NanoSizer ZS2000 (UK). The chemical composition of Fe_3O_4 was characterized by Fourier transform infrared spectroscopy (FTIR; Nicolet-20DXB, USA). Spectra were recorded over the wavenumber range of 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}. 32 scans were taken for each spectrum. X-ray diffraction pattern was performed by a X'Pert-Pro MPD diffractometer (PANalytical, The Netherlands) with CuKα radiation (λ = 0.154 nm) in wide-angle (5-90 degrees). Magnetic property of Fe_3O_4 was measured at RT using a vibrating sample magnetometer (model 7410, Lake Shore, USA).

Molecular weight of SF and SilMA was measured by gel permeation chromatography (GPC, Agilent 1260, USA). The solution concentration was 1.00 mg/ml; the injection volume was 40 microliters; and the flow rate was 1ml/min. Crystalline degree analysis of the prepared SF and SilMA coating was determined by X-ray diffractometer (D/ Max2200PC, Rigaku, China) equipped with Cu Kα radiation (wavelength of 0.1542 nm), produced at 30 kV and 30 mA. in a step-scan mode in the 2θ range of 5−90° with a scanning speed of 6°/min.

To characterize the effect of the shear mechanical properties of the SFMA coating on Ti surface, a rheological test was performed.[4] A round SFMA coating sample were tested on a DHR-2 rheometer (TA Instruments, Waters Ltd., USA). The SFMA coating specimens were a diameter of 25 mm round. The amplitude sweep was performed in

the range of 0.1%-100% strains to achieve the yielding point of the coating and the storage modulus (G') and loss modulus (G"). Frequency sweep was conducted in the range of 0.1−100 Hz at a constant oscillation strain of 1% to obtain the G' and G". All experiments were performed at 25 °C and repeated three times.

Surface morphology of Fe_3O_4/SFMA-Ti was observed by SEM. Samples were sputtered with a thin gold layer before observation. For cross-sectional view, Fe_3O_4/SFMA was coated on a silicon wafer according to the same procedure described above and then the wafer was cut into two pieces by a diamond from the middle of the wafer. Surface roughness of Fe_3O_4/SFMA-Ti was characterized using atomic force microscopy (AFM, Bruker Dimension ICON, Germany) and data was analyzed using Nanoscope analysis 14 software. The hydrophobicity of Fe_3O_4/SFMA-Ti was characterized by a water contact angle test (Dataphysics OCA20, Germany).

The exploration of SFMA composition. SFMA films was prepared by dropping 100 uL of the mixture of SF and SilMA solutions (50% w/v) in a rubber mould (8×8×2 mm length×width×height), followed with UV irradiation for 30 s and drying at 37^{o}C. The weight ratio between SF and SilMA in SFMA was varied from 10: 0 to 9: 1, 7: 3 and 5: 5 m/m, respectively. Then the SFMA films were immersed in PBS (pH 7.4) at 37^{o}C for 10 min. The shapes of the SFMA films before and after immersion were recorded using a camera to take real pictures. The expansion rate of each SFMA film was calculated according to

$$\text{Expansion\%} = A_{\text{before immersion}}/A_{\text{after immersion}} \times 100\% \quad (1)$$

Where A represents the surface area of each film as calculated from the real pictures.

Magnetic controllability of Fe_3O_4/SFMA on Ti. Fe_3O_4/SFMA films in different SF/SilMA composition were coated on Ti discs as described above and then immersed in PBS (pH 7.4) for 24 h at 37^{o}C in a 24-well plate. After immersion, a NdFeB magnet (1 cm diameter, 1 mm height, 1.17–1.21 Tesla residual magnetism) was put beneath the well and moved around the Ti disc for 1 min to magnetically pulled Fe_3O_4 nanoparticles off the Ti surface. After pulling-off, the liquid in each well was removed and 0.5 mL of aqua regia that consisted of nitric acid (15M) and hydrochloric acid (12M) in a ratio of 1:3 v/v was added to dissolve the remained Fe_3O_4 nanoparticles on each surface. The

amount of the remained Fe_3O_4 nanoparticles was quantified by measuring the concentration of iron ions using inductively coupled plasma-mass spectrometry (ICP-MS; Agilent 7500a, Hewlett-Packard, Yokogawa Analytical Systems, Tokyo, Japan) according to

$$\text{Remained } Fe_3O_4\ \% = C_{Fe3+\text{ after pulling-off}}/C_{Fe3+\text{ before pulling-off}} \times 100\% \quad (2)$$

Where C represents the concentration of Fe^{3+} of each sample.

Calculation of the relationship between the saturated magnetic strength of the externally-used magnet and its maximum working distance. The magnetic traction force of the externally-used magnet to Fe_3O_4/SFMA coating was calculated by a computer simulation using magnetostatic module in Ansys 2019R3 software (Fig. S6).[5] The Fe_3O_4/SFMA coating was considered to be a 5-μm thick layer of Fe_3O_4 disc with a diameter of 10 mm (Fig. 4a). The shape of the applied NdFeB magnet was set with a height of 2.5 mm and a diameter of 10 mm. The coercive force and the residual induction of the Fe_3O_4/SFMA coating was set as 36000 (A /m) and 0.01 Tesla. The coercive force of the applied NdFeB magnet was set as 8.11×10^5 (A/ m) and its residual induction was varied from 1 to 2 and 3 Tesla, respectively. The distance between the magnet and the externally-used magnet to Fe_3O_4/SFMA coating is varied from 0.5 to 1.0, 2.5, 5.0, 7.5 and 10 mm, respectively (Fig. 4b). The magnetic traction force of the externally-used magnet to Fe_3O_4/SFMA coating at different distance was calculated and a standard curve was then fitted. The distance at which the magnetic traction force reached 0.1 N was considered as the maximum working distance according to the standard curve.

Adhesion force and stability of Fe_3O_4/SFMA on Ti. The adhesion force of Fe_3O_4/SFMA (in a SF/SilMA ratio of 5:5) coating on Ti surface was measured by a scratch test using a nano/micro-indenter (NHT³, Anton Paar, Austria). Briefly, a 2-mm length scratch was done by an indenter (100 um in diameter) in a speed of 2 mm/min. The loading force of the indenter increased from 0.03 N to 5 N and the friction that the indenter bore from the coating was recorded as a function of the displacement. Coating stability of Fe_3O_4/SFMA on Ti surface was tested by immersing the Ti discs in PBS (pH 7.4) for 8 weeks. After drying at 37°C, surface morphology was observed by SEM.

For quantification, Fe_3O_4/SFMA coatings on Ti discs were dissolved by aqua regia and the concentration of Fe^{3+} in each solution was measured using ICP-MS and the remained Fe_3O_4 on Ti surfaces after immersion was calculated according to

$$\text{Remained } Fe_3O_4\ \% = C_{Fe3+\text{ after immersion}}/C_{Fe3+\text{ before immersion}} \times 100\% \quad (3)$$

Where C_{Fe3+} denotes the concentration of Fe^{3+} after dissolving Fe_3O_4 from Ti surfaces.

***In vitro* biofilm treatment on Fe_3O_4/SFMA surface.** For bacterial culturing and harvesting, Methicillin-resistant *Staphylococcus aureus* ATCC 43300 (MRSA) and *Escherichia coli* ATCC 25922 (*E. coli*) were grown from frozen stock ((7 v/v% dimethyl sulfoxide / Tryptone Soy Broth (TSB; OXOID, Basingstoke, UK)) on TSB agar plates at 37°C for 24 h. Bacteria from a single colony were suspended in TSB and incubated for 16 h at 37°C in an oscillating incubator (190 rpm). Bacteria were harvested by centrifugation (5000 ×g, 5 min, 10°C) and washed twice with PBS (pH 7.4). Bacterial suspension was then sonicated 3 times (30/15 s on/off) at 0°C in an ice/water bath to get single bacteria. Bacterial concentration was determined using UV spectrophotometer (Shimadzu, Japan) at 650 nm according to a calibration curve measured before[3] and diluting into a certain value for later use.

For biofilm treatment, mature MRSA and *E. coli* biofilms were built on Fe_3O_4/SFMA surfaces and followed with antibiotic killing in the presence or absence of magnetic pulling-off Fe_3O_4 nanoparticles from the Fe_3O_4/SFMA surfaces. Briefly, Fe_3O_4/SFMA (in a SF/SilMA ratio of 5:5) coated Ti discs were place in a 48-well plate. 500 uL of bacterial solution in a concentration of 10^9 CFU/mL was added into each well and placed for 2 h at RT for bacterial adhesion on Fe_3O_4/SFMA surfaces. Then the liquid was removed and the surfaces were gently rinsed with PBS (pH 7.4) for 3 times to remove the non-attached bacteria. Another 500 uL of TSB growth medium was added into each well and the well plate was kept at 37°C for 48 h to ensure a mature biofilm growth. The medium was refreshed every 24 h. After 48 h, liquid in each well was removed and 500 uL of antibiotic solution in PBS (pH 7.4) was added and an external magnet was used to magnetically pull the Fe_3O_4 nanoparticles off the surface. Specifically, the magnet was firstly putting beneath the well palte and moving around

for 1 min and then the magnet was putting above the well for another 1 min to pull the Fe_3O_4 nanoparticles off. For MRSA biofilm treatment, vancomycin in a concentration of 200 ug/mL was used while for *E. coli* biofilm treatment, gentamicin 250 ug/mL according to the minimum bactericidal concentration (MBC) value that measured in Table S1. The well plate was kept at 37°C for another 24 h and the killing efficacy was detected by CFU enumeration. Biofilms treated by PBS (pH 7.4) in the absence of magnetic pulling-off were considered as blank control.

For confocal laser scanning microscopy (CLSM) visualization, biofilms after above treatments were stained with green-fluorescent SYTO9 (Thermo Fisher Scientific, Waltham, MA) and red-fluorescent propidium iodide (PI, Thermo Fisher Scientific, Waltham, MA) for 15 min at RT in the dark. Then biofilms were washed once with PBS (pH 7.4) and subsequently imaged by CLSM (Leica TCS SP2 Leica, Aqueouszlar, Germany) with an HCX APO L40×/0.80 W U-V-1 objective. An argon ion laser at 488 nm and a green HeNe laser at 543 nm were used to excite the SYTO9 and PI, and fluorescence was collected at 500−540 nm (SYTO9) and 583−688 nm (PI). CLSM images were acquired using Leica software, version 2.0.

For SEM observation, biofilms after above treatments were fixed with glutaraldehyde (2.5%) for 30 min at 4°C and dehydration in a graded ethanol series (50, 60, 70, 80, 90, 100%) for 10 min each. All samples were dried overnight in a vacuum oven and sputter coated with a thin gold layer before observation.

***In vitro* biocompatibility of Fe_3O_4/SFMA.** For cell culture, the Mouse embryonic osteoblast precursor cells (MC3T3-E1) were cultured in α-minimal essential medium (α-MEM, Gibco, Grand Island, NY, US) supplemented with 10% of fetal bovine serum (FBS, Hyclone, USA), 100 U/mL of penicillin G and 100 mg/mL of streptomycin in a cell incubator (100% relative humidity, 5% CO_2, 37°C). After the culture achieved approximately 90% confluence, cells were digested with 0.25% trypsin/EDTA solution and re-suspended in fresh culture medium. For evaluation *in vitro*, sample extracts were prepared using α-MEM supplemented with 10% fetal bovine serum (FBS) for 24 h at an extraction ratio of 1.25 cm^2/mL.

Cell viability in sample extracts was evaluated using a cell counting kit (CCK-8, HY-K0301, MedChemExpress, USA) test. MC3T3-E1 cells were cultured in 0.1 mL of medium in a 96-well plate (5,000 cells per well) for 24 h. Then the medium in each well was replaced by sample extracts. After culturing for 1, 3 and 5 days, fresh cell medium supplied with 10% CCK-8 was used to replace the medium and the cultures were further incubated for another 1.5 h. The absorbance of each well after formazan generation was detected using a microplate reader (1681130, Bio-Rad, USA) at λ= 450 nm and the cell viability of each sample was calculated according to

$$\text{Cell viability (\%)} = (A_{sample} - A_{negative})/(A_{positive} - A_{negative}) \times 100\% \quad (4)$$

Where A_{sample} or $A_{positive}$ represents the absorbance value of the well in which cells were incubated by sample extract or medium, $A_{negative}$ represents the absorbance value of the well in absence of cell.

Cell toxicity of sample extracts was studied by a live/dead staining assay. MC3T3-E1 cells were cultured in 0.5 mL of medium in a 48-well plate (20,000 cells per well) for 24 h. Then the medium in each well was replaced by sample extracts. After culturing for 24 h, cells were washed with PBS (pH 7.4) for three times and followed with calcein/PI cell activity and cytotoxicity assay kit (C2015M, Beyotime Biotechnology, China) staining. Finally, cells in each well were observed using a fluorescent microscope (BX-51, Olympus, Japan) at excitation wavelengths of 494 and 535 nm.

To observe the effect of sample extracts on cell morphology, MC3T3-E1 cells were cultured in 1.0 mL of medium in a 24-well plate (20,000 cells per well) for 24 h. Then the medium in each well was replaced by sample extracts. After culturing for 24 h, cells were washed with PBS (pH 7.4) for three times, fixed by 4% w/v of paraformaldehyde for 10 min, and treated with 0.5% v/v of Triton X-100 for another 10 min. Then cellular actin was stained by 1.0% v/v of FITC-phalloidin (P5282, Solarbio, China) for 90 min at RT and the cell nuclei was stained by 1mg/mL of DAPI (4,6-diamino-2-phenylalanine, Solarbio, China) for 10 min at RT. Finally, samples were washed with PBS (pH 7.4) for three times and observed and analyzed under a CLSM at excitation wavelengths of 488 and 405 nm.

***In vivo* IAIs treatment on Fe_3O_4/SFMA surface in a rat sub-cutaneous model.**

The rat experiment was approved by the Ethics Committee, Peking University Health Science Center, Beijing, China. Procedures were in accordance with requirements established by the Experimental Animal Ethics Branch (PUIRB-LA2023093). Male Sprague-Dawley rats (7 weeks old, 200-250 g, purchased from Vital River Laboratory Animal Technology Co., Beijing, China) were randomly divided into three groups: PBS, Vancomycin and Vancomycin + pulling-off (n=6). Prior to surgery, SD rats were anesthetized with pentobarbital sodium (50 mg/kg). After anesthetization, the dorsal hair of rats was removed using a razor. A skin wound (1 cm in length) was created using a surgical blade under sterile condition on the back of each rat. Fe_3O_4/SFMA coated Ti discs were implanted sub-cutaneously and the wounds were then sutured. 100 uL of MRSA suspension (10^9 CFU/mL) in PBS (pH 7.4) was injected on the surface of each Ti disc to cause biofilm infection. After 48 h, 100 μL of vancomycin in PBS (10 mg/mL yielding 120 mg vancomycin per kg body weight) was injected locally on Fe_3O_4/SFMA-Ti surface. A NdFeB magnet that used above was then put on the infected site and moved around for 5 min. After 3 days, half of the rats were euthanized by overdosed pentobarbital sodium and the rest were administrated with another 100 μL of vancomycin and then sacrificed at day 7. Tissues that contacted with the Ti disc surface were excised, weighed and homogenized with sterile PBS (pH 7.4) for CFU enumeration. Bacteria on Ti discs were detached under sonication and then countered by CFU enumeration. For SEM observation, Ti discs were fixed with glutaraldehyde and dehydrated with ethanol according to the same procedure as described above. For hematoxylin and eosin (H&E), Masson and Immunohistochemical staining, excised tissues were kept in 4% paraformaldehyde without homogenization. Blood was collected from three rats that were sacrificed at day 7 for blood chemistry, including levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and urea nitrogen (BUN) that indicates possible influences of the treatments on liver and kidney function and routine blood parameters, including levels of white blood cells (WBC), red blood cells (RBC), platelets (PLT), hemoglobin (Hgb), hematocrit (HCT), mean

corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH).

Statistical analysis. Data was expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) with student's t-test was performed for statistical significance among groups. *$p < 0.05$ was considered to be statistical significance.

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