

Supporting Information

A smart indwelling needle with on-demand switchable anticoagulant and hemostatic activity

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Experimental Section

Materials: Chitosan (CHI, 75% deacetylated), 3,4-dihydroxyhydrocinnamic acid (HCA) 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) and dopamine hydrochloride (DA) were purchased from Sigma-Aldrich. Heparin sodium salt (Hep, MW 15,000 Da) was purchased from Tianjin Dingguo Biotechnology Co. Ltd. Polylysine (PLL) modified ferric oxide nanoparticles (for simplicity, FeNPs was used to denote PLL-modified ferric oxide) were supplied by Nanjing XFNANO Co. Ltd. -

Synthesis of CHCS: CHCS was synthesized according to previous reports.^{1, 2} Briefly, chitosan (1g) was dissolved in HCl solutions (0.1 M, 50 mL) and then the pH of the solution was adjusted to 5.0-5.5 with 1 M NaOH. The final concentration of chitosan was set at 1 wt%. Then, 3,4-dihydroxy hydrocinnamic acid (0.6 g) was added, followed by adding EDC (1:1 HCA/EDC molar ratio, dissolving in 20 mL 50% ethanol aqueous solution) dropwise into the reaction mixture. The coupling reaction was performed at room temperature for 4 h (pH 5.0) under vigorous stirring. Immediately after the reaction, the mixture was dialyzed in acid solution (HCl, pH 5.0) for two days, in PBS solution for 4 h, and in ultrapure water for 24 h to remove the unreacted agent. The final product was lyophilized to obtain the resultant CHCS.

Synthesis of HepDA: Heparin (1 g) was dissolved in 0.1 M MES buffer (pH5.5, 100 mL) and then NHS (5 mM, 0.115 g) and EDC (10 mM, 0.383 g) were added to the solution. The mixture was stirred at room temperature for 1 h to activate the carboxylic acid groups of heparin, followed by adding an aqueous solution of dopamine (0.3 g) in MES buffer. The mixture was vigorously stirred overnight at room temperature. After completion of the reaction, the solution was transferred to a dialysis membrane (MWCO = 3500 Da), and dialyzed against acidified water (0.01 M HCl) for 24 h to remove unreacted dopamine and coupling reagents. The final product was collected by lyophilization and named as HepDA. The relative amount of dopamine in HepDA was

estimated by UV-vis spectrum based on the intensity of benzene's absorption peak (280 nm).

Preparation of Hemostatic-Anticoagulant Indwelling Needle: First, the DA solution (dissolved in 10 mM Tris buffer, pH 8.5, 5 mg/mL) was injected into the cannula from the pinhead of the indwelling needle (24G), and maintained at 37 °C for 4 h to allow the self-polymerization of dopamine to form PDA primer. After that, the FeNP-HepDA solution (5 mg/mL) were injected into the cannula and incubated at 37 °C for 12 h. Then the inner surface of the indwelling needle was washed for three times with deionized water for 5 min, followed by drying in vacuum. For preparation of hemostatic coating on the outside surface, the CHCS was dissolved in the deionized water and ethanol solution (1.5 wt%) and incubated at 4 °C for three days to subject to the partial oxidation. Subsequently, the partial oxidized CHCS solution was gently dropped onto the external surface of the cannula of the indwelling needle. The solution was completely dried at room temperature. Similar procedure was performed on the surface of tetrafluoroethylene plate to mimic the modifications of CHCS and FeNP-HepDA coatings on the indwelling needle.

Characterization: The surface roughness before and after FeNP-HepDA immobilization onto PTFE substrate was evaluated by atom force microscopy (AFM) (Agilent 5500, USA). The change of functional groups of CHCS and HepDA was measured by Fourier transform infrared spectroscopy (FTIR, Nicolet 6700) over a wavenumber range from 400 to 4000 cm^{-1} . The UV-vis spectrum was determined in the range from 200 to 400 nm by TU-1810 UV-vis spectrophotometer. Static water contact angle on PTFE substrate modified with different materials were obtained using a JC2000D contact angle meter (Powereach, China) at room temperature. The average of three measurements on each sample was taken at different sites. The morphology of the hemostatic-anticoagulant needles were observed using SEM (Hitachi S-4800). The distribution of different atomic species onto the internal and external surface of indwelling needle was measured by energy-dispersive spectroscopy (EDS) in tandem with SEM.

In Vitro Stability of Modified Coating: To measure the stability of FeNP-HepDA in hemostatic-anticoagulant needle under magnetic field in a physiological environment, the hemostatic-anticoagulant needle taped with a magnet was immersed in PBS solution (pH 7.4) at 37 °C with shaking at 100 rpm. An equal volume of fresh buffer was added when the release solution was removed. The concentration of HepDA was measured at 263 nm using UV-vis spectrum. The bare needle sealing with same dosage of FeNP-HepDA was set as a control group.

Platelet Adhesion: The surface thrombogenicity of the samples was evaluated using platelet adhesion test. Platelet rich plasma (PRP) was obtained by centrifuging anticoagulant fresh whole blood at 1500 rpm for 15 min. Then 1 mL PRP was dropped onto the surface of PTFE substrates with different surface modification and incubated at 37 °C for 30 min. The bare PTFE substrate was employed as a negative control. After that, the substrates were carefully rinsed three times with PBS for 3 min to remove nonspecifically adhered platelets. 2.5 % glutaraldehyde was then used to fix the samples for 12 h at room temperature, followed by adding 30%, 50%, 70%, 80%, 90% and 100% ethanol solution for dehydration. After critical point drying with CO₂, the substrate surface was observed by SEM.

In Vivo Evaluation of Anticoagulation and Hemostasis: All the protocols for animal experiments were carried out according to the guidelines of the Council for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Public Health, China. The animal experiments were approved by the Animal Ethical Committee of Institute of Hematology, Chinese Academy of Medical Sciences, China. New Zealand white rabbits (male, 12–14 weeks old, n = 6) were used for the animal model to evaluate the in vivo anticoagulation and hemostasis of the modified indwelling needles. All animals were chosen at random and the investigator was blinded to the groups in all experiments. Hair from the ear of each rabbit was first shaved with electric clipper, and then the rabbit was anaesthetized by breathing isoflurane with inhalational anaesthesia systems. The modified indwelling needle was inserted into rabbit's marginal ear vein and fixed with adhesive bandages. For magnetic response group,

magnet (0.1T) was stuck to the skin surface of the rabbit's ear where the indwelling needle cannula was located. Blood was drawn from the needle every 30 min, followed by injecting saline to wash residual blood on the internal side of the catheter. After 3 h implantation, the needles were gently withdrawn from the blood vessel. When no blood could be drawn from the blood vessel (meaning blood coagulation near the cannula), the needle was withdrawn immediately. The blood loss after withdrawal of needles was calculated by using cotton ball to absorb the spilled blood.

Cytotoxicity Assay: CCK-8 was used to evaluate the cell cytotoxicity of the nanoparticles and polymers used in this experiment. Human umbilical vein endothelial cells (HUVE) suspension (100 μ L) was added into 96-well microplates, with 5000 cells being immersed in the complete growth medium per well, cultivated in a humidified 5% CO₂ atmosphere at 37 °C for 24 h for cell attachment. Subsequently, FeNPs, HepDA, CHCS and FeNP-HepDA (10 μ L) at a concentration of 1 mg/mL were respectively added to 96-well plates, followed by incubation for 24 h. Then, CCK-8 solution was added to 96-well plates at 10 μ L per well and incubated for 3 h. The resulting solutions were analyzed at 450 nm on a plate reader (BIO-TEK instruments Inc EL311S, America). This process was repeated for 8 times in parallel. The results were expressed as the relative cell viability (%) with respect to blank group only with culture medium. The cell viability in each well was calculated from the obtained values as a percentage of control wells.

Hemolysis Assay: Fresh blood was obtained from a rabbit, whose erythrocytes were separated by centrifugation at 3000 rpm for 10 minutes, washed three times with saline solution, and finally diluted with erythrocyte stock (100 μ L, 5%). The samples of FeNPs, HepDA, CHCS and FeNP-HepDA was added to the solution, respectively, and incubated for 1 h at 37 °C. Then the samples was centrifuged at 1000 rpm for 5 minutes and the supernatants were collected for further detection. Hemolytic activity was determined by OD_{545nm} using a multifunctional microplate reader. Red blood cells (RBC) in saline solution was a negative control and deionized water was a positive

control. The hemolysis percentage (Hemolysis %) was calculated from the following equation:

$$\text{Hemolysis\%} = [(A_S - A_-) / (A_+ - A_-)] \times 100\% \quad (2)$$

where A_S is the absorbance of the samples, A_- is the absorbance of the negative control and A_+ is the absorbance of the positive control.

APTT and PT Test: Activated partial thromboplastin time (APTT) and thrombin time (TT) were monitored to examine the influence of the sample surface on the coagulation system. Briefly, the indwelling needle with various coatings was immersed into 500 μL platelet-poor plasma (PPP) and incubated at 37 °C for 30 min. For the APTT test, 100 μL incubated PPP was transferred to the test tube, followed by adding 100 μL APTT agent and incubated at 37 C for 3 min; subsequently 100 μL 0.025 M CaCl_2 was added and the clotting time was measured in an automatic blood coagulation analyzer For PT test, 100 μL PT reagent was added to the test tube, followed by adding 100 μL incubated PPP and then incubated at 37 C for 3 min. Next, the clotting time was measured using an automatic blood coagulation analyzer.

Biochemical Analysis: The rabbit's serum samples of treatment group and control group (normal rabbit) were analyzed for a wide range of general biochemical parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine. These parameters were measured with standard automated biochemical analyzers. In this test, 3 rabbits were used.

Statistical Analysis: Both *in vitro* and *in vivo* experiments were analyzed by one-way student's t-test and expressed as means \pm standard deviations (SD). Statistical significance was defined as having $*P < 0.05$. SPSS 20.0 was used for statistical analysis of data.

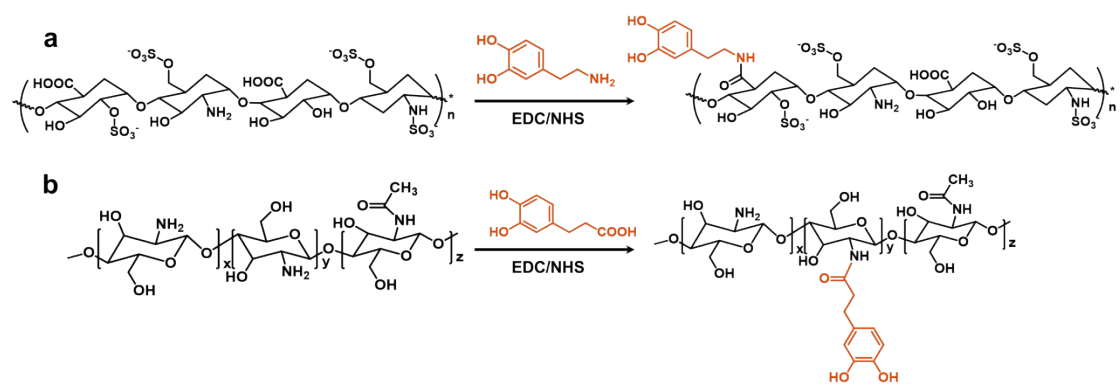


Fig. S1 Synthetic route of heparin–dopamine (**a**) and catechol-conjugated chitosan

(**b**).

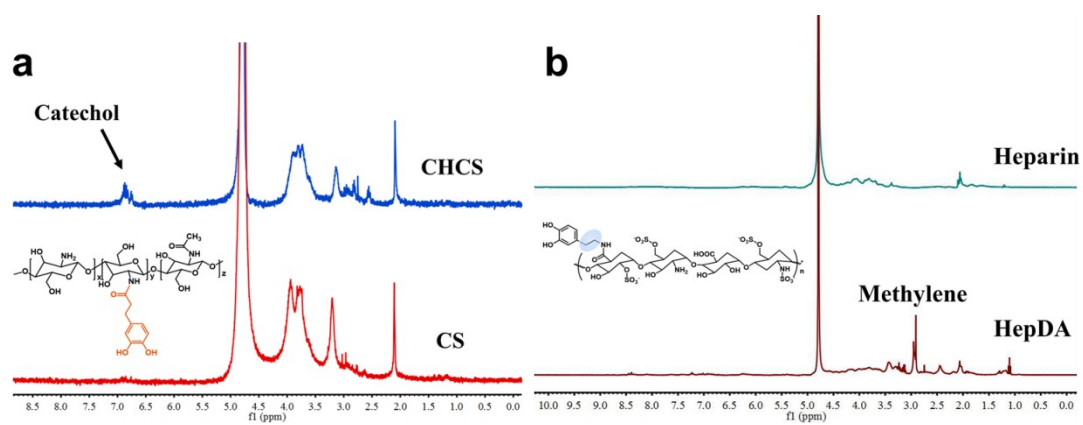


Fig. S2 ^1H -NMR spectra of CS, CHCS (a) and heparin, HepDA (b).

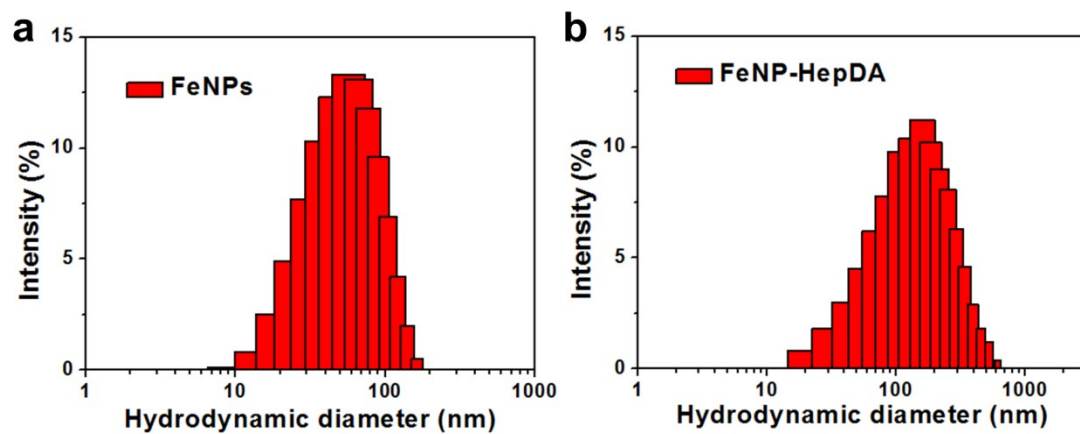


Fig. S3 Hydrodynamic diameters and distributions of FeNPs (**a**) and FeNP-HepDA (**b**).

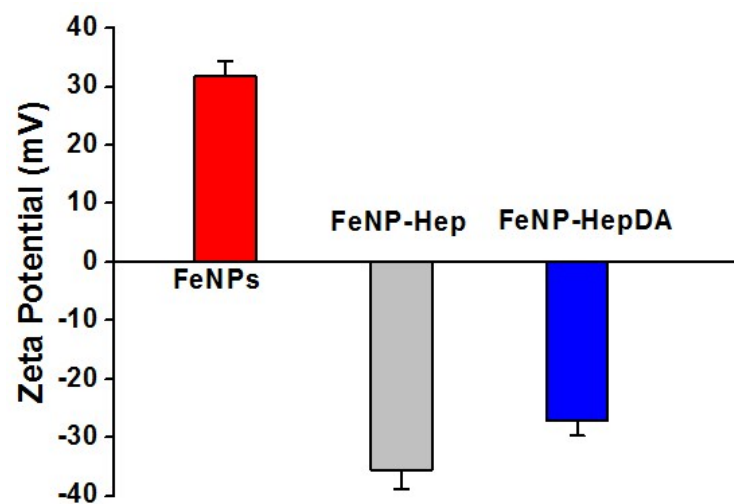


Fig. S4 Variation of zeta-potentials of FeNP-Hep and FeNP-HepDA during coating process of heparin and HepDA.

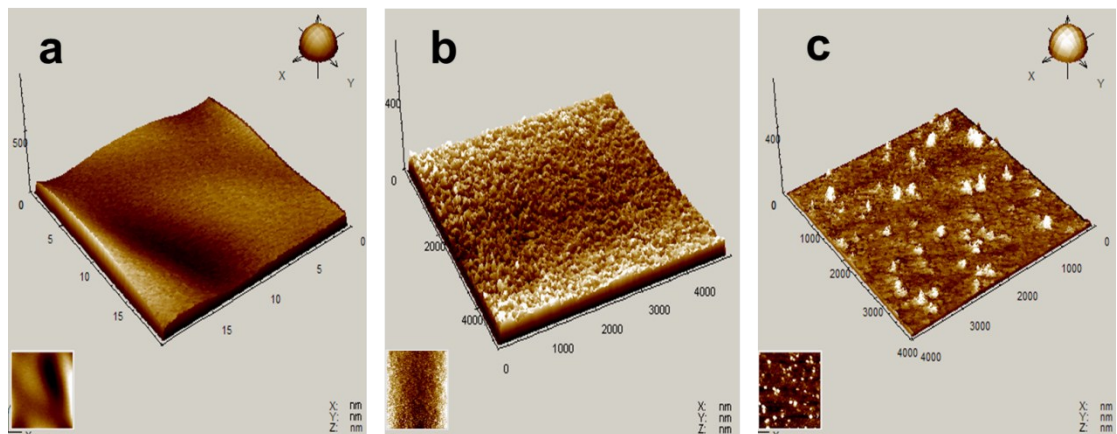


Fig. S5 AFM images of PTFE substrate (a) and FeNP-HepDA immobilized PDA-coated PTFE in the presence (b) and absence (c) of a magnetic field.

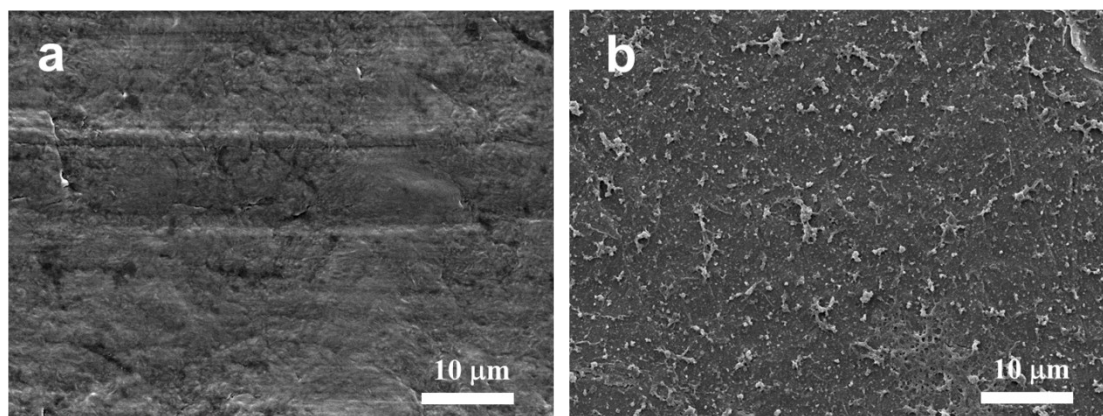


Fig. S6 SEM images of PTFE before (a) and after (b) immobilization of FeNP-HepDA.

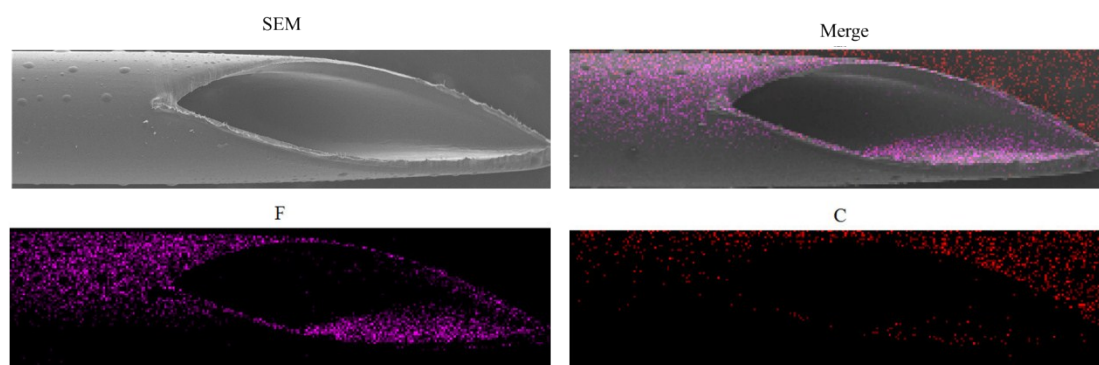


Fig. S7 SEM images and EDS analysis of bare indwelling needle.

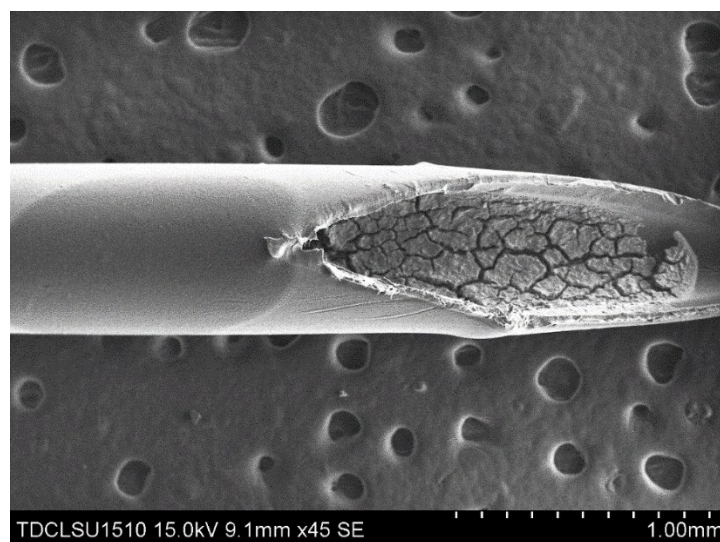


Fig. S8 SEM image of FeNP-HepDA-modified indwelling needle without pre-coating of PDA.

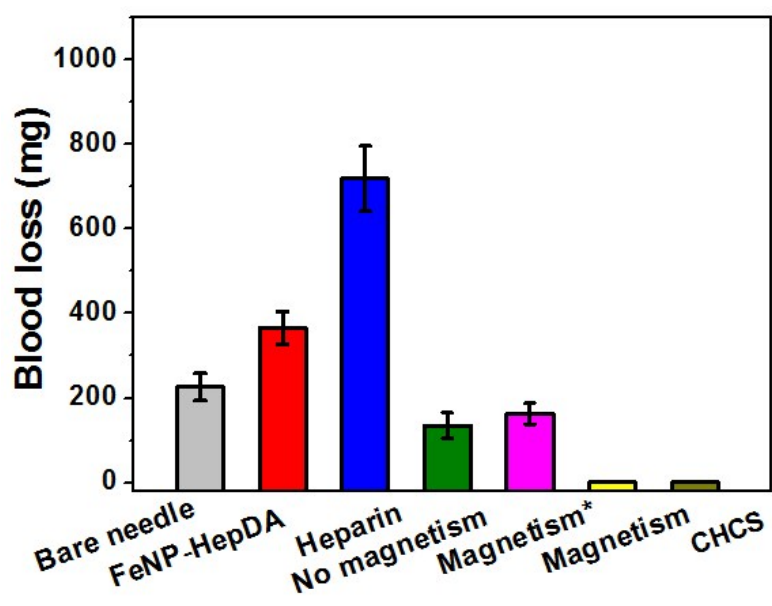


Fig. S9 Quantitative analysis of blood loss after the extraction of the indwelling needle with different modification methods. Asterisks (*) denoted significant differences ($P < 0.05$). Magnetism* means the doubly modified indwelling needle was directly pulled out under magnetic field.

	10 min	0.5 h	1 h	1.5 h	2 h	2.5 h	3 h
Group 1	⌘						
Group 2	⌘	⌘	⌘	⌘	⌘	⌘	
Group 3	⌘	⌘	⌘	⌘	⌘		
Group 4	⌘	⌘	⌘	⌘	⌘	⌘	⌘
Group 5	⌘	⌘	⌘				
Group 6	⌘						

Table S1 The blood clotting time of various treatment groups. ⌘ means coagulation of blood; ⌘ represents the patency of indwelling needle.

Index	Alanine aminotransferase (ALT) (U/L)	Aspartate aminotransferase (AST) (U/L)	Creatinine (μ mol/L)	Urea (mmol/L)
Treatment group	52.6 \pm 8.1	27.2 \pm 8.6	81.9 \pm 8.5	8.4 \pm 1.2
Control group	58.4 \pm 10.9	27.2 \pm 6.2	76.2 \pm 14.7	8.0 \pm 3.2

Table S2 The function indices of liver and kidney including ALT, AST, creatinine, urea of treatment group and control group based on biochemical analysis. The normal rabbit and the rabbit treated with doubly modified indwelling needle was set as the control group and treatment group, respectively. There was no significant difference between treatment group and control group based on one-way student's t-test statistical analysis.

The representative movies for different groups of indwelling needles in rabbit model were supplied.

Movie 1: Bare needle

Movie 2: Bare needle sealing with heparin

Movie 3: Indwelling needle modified with FeNP-HepDA on inner surface and CHCS on outside surface under magnetic field

References

1. K. Kim, K. Kim, J. H. Ryu and H. Lee, *Biomaterials*, 2015, **52**, 161-170.
2. M. Shin, S. G. Park, B. C. Oh, K. Kim, S. Jo, M. S. Lee, S. S. Oh, S. H. Hong, E. C. Shin, K. S. Kim, S. W. Kang and H. Lee, *Nat. Mater.*, 2017, **16**, 147-152.