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Hyperthermia triggered UK release nanovectors for deep venous thrombosis therapy

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Abstract

Deep vein thrombosis (DVT) is a common and lethal complication of surgery. In clinic, thrombolytic drugs are primarily used for treating DVT. However, th e utilization of thrombolytic drugs is limited due to the risk of urokinase (UK) -related hemorrhagic complications. In this paper, a binary eutectic phase-chang e fatty acid composed by lauric acid and stearic acid was used to block the p ores of gold-mesoporous silica core-shell nanoparticles, so as to deliver thromb olytic drugs. The eutectic mixture has a well-defined melting point at 39.2 °C, which can be used as a biocompatible phase-change material for hyperthermia-t riggered drug release. The prepared system presents remarkable photothermal ef fect owning to the gold nanoparticles and quick drug release in response to ne ar-infrared irradiation (NIR). In addition, the localized hyperthermia also could enhance the lysis of thrombus. The thrombolytic effect of this system was eval uated in vitro and in vivo. Hereinto, rabbit femoral vein thrombosis model was firstly built for imitating thrombolysis in vivo. The B-ultrasound was then used to monitor the changes of thrombus after treatment. The results presented that the reported system could be potentially used to deliver thrombotic drugs in cli nic.

Keywords

Fatty acid; Urokinase; Thrombus; Gold nanoparticle; Thrombolysis

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Introduction

The incidence of DVT in the population is 1 per 1000. Common risk factors for DVT include senile, obesity, brake, malignancy, previous DVT history, oral contraceptives, substance abuse, recent surgery and acute infectious disease[1, 2]. The embolus falls off and enters the bloodstream to the distant place, which leads to the occurrence of myocardial infarction, cerebral infarction, pulmonary embolism and may cause long-term disease due to post-thrombotic syndrome[3]. The disease of DVT is very harmful, timely successful dissolution of intravascular blood clots and lysis of the fibrin network are critical for tissue survival and vascular function recovery. It is a significant strategy that involves systemic or transcatheter administration of fibrinolysis/thrombolytic agents for medication such as UK[4]. UK is suitable for patients with thromboembolism in the acute phase without thrombolysis contraindications. But currently, the thrombolytic drugs used in clinical practice have short half-life and some serious side effects (such as bleeding)[5]. Therefore, how to deliver thrombolytic drugs to the thrombus safely and effectively is a urgent problem to be solved.

The development of nanotechnology provides a new strategy for the safe delivery of drugs. Nanoscale drug delivery system has become a hot topic in today's research. Photothermal therapy (PTT) using light absorber to locally produce excessive heat under near-infrared irradiation has great clinical application prospects for tumor replacement therapy[6-8]. UK-loaded nanoparticles (NPs) reduce side effects while increasing the benefits of thrombolytic[9, 10]. Herein, for lower extremity deep vein thrombosis, we constructed a new thrombolytic drug delivery system based on fatty acid and mesoporous silica-gold shell-core structure. The system can trigger the photothermal effect of gold nanoparticles under the irradiation of NIR[11, 12], and then cause the fatty acid to undergo a phase change reaction, thereby releasing the thrombolytic drug encapsulated in mesoporous silica. In addition, the local hyperthermia caused by the system will also promote the dissolution of the thrombus. Due to the constant melting temperature and large latent heat, we selected the eutectic mixture fatty acids (FA) of lauric acid (LA) and stearic acid (SA) as the phase change material (PCM) for blocking the drugs[13]. In addition, after fatty acids degradation, the decomposition products are beneficial to increase high-density lipoprotein, reduce low-density lipoprotein, and have a better effect on reducing the risk of atherosclerosis than other fatty acids[14]. In addition, the prepared system also provides

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a localized hyperthermia for enhanced thrombolysis for DVT therapy, which increases safety for topical thrombolysis, and creats a new and promising treatment (Figure 1). In this paper, we evaluated the thrombolytic effect and cell safety of the system in vitro. More importantly, we firstly evaluated the thrombolytic effect of the system in the rabbit femoral vein model for lower extremity deep venous thrombosis.

Results and Discussion

2.1. Preparation and Characterization of the Designed System

First of all, we synthesized gold@mesoporous silica core–shell nanospheres (Au@MSNs) through the classic one-step method[15]. Scanning electron microscope (SEM) images showed the morphology of Au@MSNs(Figure 2A). Next, the eutectic mixture of LA and SA can be added as a plugging material for mesoporous silicon[16]. The synthesis of UK-FA@UK-FA@Au@MSNs was further confirmed from the image of transmission electron microscope (TEM) (Figure 2B; Figure S1, Supporting Information)：After CTAB templating agent was removed, it could be seen that the surface of the sphere was rough, indicating the presence of mesoporous silicon. The measured Brunauer–Emmett–Teller (BET) surface area was445.37779 m^2/g with a pore volume of 1.143 cm^3/g and pore size of 10.267 nm (Figure 2C). The dynamic light scattering (DLS) results of Au@MSNs were presented in Figure 2D. The results of Fourier transform infrared spectra showed that the infrared spectrum morphology of Au@MSNs after loading urokinase was basically the same as that before loading，but there were new peaks. The new peaks represented the stretching vibration of C-H (2931.316 cm^{-1}) and the transformative vibration of CH_2 (1390.447 cm^{-1}) (Figure S2, Supporting Information). The Fourier transform infrared spectra results mainly confirmed that the outermost fatty acid can be well blocked on the surface of mesoporous silicon. From Figure 2E, for in vitro release study, in the first 20 min, 64.42% of the UK was released rapidly from UK-FA@Au@MSNs with NIR group, especially in 10-20 min, the curve was very steep. The accumulative release rate of the drug reached 81.46% after 30 min. In the UK-FA@Au@MSNs without NIR group, UK was almost undetected throughout the whole time course. In order to further study the temperature increasing effect of the material in the local part, as shown in Figure 2F, under the NIR, the heating rate increases with the increasing concentration, and when UK-FA@Au@MSNs was 1.0 mg/mL, it could be quickly reached up to 39.2 °C (2 W/cm^2).

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2.2. In vitro plaque dissolution

As shown in Figure 3A, in the absence of flow[17], the clot lysis can be carefully observed in four groups:Control group(Blood clots without treatment), NIR group(Blood clots deal with near-infrared irradiation), UK group(Blood clots deal with UK), UK-FA@Au@MSNs with NIR group(Blood clots deal with UK-FA@Au@MSNs under the near-infrared irradiation). After one hour, in the NIR group, the UK group and the UK-FA@Au@MSNs with NIR group, the color of the solution was gradually deepened. In contrast, two hours later the color of the control group remained unchanged. Figure 3B showed the blood clot lysis in the presence of flow by the same groups as above. Under observation, the time of blood dissolution was separated. The thrombolysis was not observed in the control group. The dissolution time of the UK group was nearly 70 minutes, which was slightly better than the NIR group for nearly 90 minutes. However, the UK-FA@Au@MSNs group with NIR showed a minimum thrombolysis time about 40 minutes, showing blood flow time was associated with near-infrared controlled release, and indicating that synthetic nanoparticles could be used for temperature sensitive delivery, which could improve thrombolytic therapy.

2.3. Cell experiment

Before we begin to analyze thrombolysis in vivo, it is important to reveal that the synthesized nanoparticles have low cytotoxicity. Firstly, to assess Human Umbilical Vascular Endothelial Cells (HUVECs) injury by UK-FA@Au@MSNs quantitatively, we examined the effects of UK-FA@Au@MSNs on endothelial lactate dehydrogenase (LDH) release, Prostacyclin (PGI_2) release, Nitric Oxide(NO) release after 24 hours by ELISA. Lactate dehydrogenase (LDH), a stable cytosolic enzyme released during cell lysis. When the concentration of UK-FA@Au@MSNs increased, the content of lactate dehydrogenase (LDH) decreased. Then the concentration reached 100ug/mL, the release amount was 0.84 times compared with the control group (Figure 4A). Vascular endothelial cells play an important role in the regulation of vasomotor function and platelet activity through autocrine Prostacyclin (PGI_2) and Nitric Oxide(NO). Nitric Oxide(NO) is considered to be a key determinant of vascular health and exerting antiplatelet, antithrombotic and anti-inflammatory properties in the vascular system[18-20]. From Figure 4B, a slight release of Nitric Oxide(NO) was reduced. When the drug concentration was 100 ug/mL, the release of Nitric Oxide(NO) was 0.80 fold than that of the control group. Prostacyclin (PGI_2) has specialities of vasodilation, antiplatelet aggregation, anti-leukocyte adherence and

cytoprotective effects[21-23]. From Figure 4C, the release amount of Prostacyclin (PGI_2) was positively correlated with the drug concentration. When the drug concentration was 100 ug/mL, the release amount of Prostacyclin (PGI_2) was 2.39 fold compared with the control group. To further certify the effect of the synthesized material on the proliferation of HUVECs, the CCK8 kit was used for detection. From Figure 4D. Interestingly, after the first and second day of incubation with the drug, the cells were slightly proliferated, and the cell viability was still close to the untreated group on the third and fourth day. It was concluded from the results that the drugs we synthesized had favorable biocompatibility and safety.

2.4. In vivo thrombolysis

The adult New Zealand white rabbit was selected to successfully establish a rabbit femoral vein thrombosis model by blocking and injecting thrombin[4]. Experiments were divided into four groups (untreated group, UK group, NIR group, UK-FA@Au@MSNs with NIR). The dose in the UK group was 2 mg and 8 mg for the UK-FA@Au@MSNs with NIR. It took 30 minutes to apply NIR and the power was $2W/cm^2$. The models were successfully administered 24 hours later. Before the injection, the B-ultrasound image was used to confirm the thrombus. After 24 hours of injection, it was the time point that began to observe the changes of thrombus formed in the first, the second and the third day. From Figure 5A, the thrombus in the treatment group was dissolved compared with untreated. The UK group dissolved better than NIR group, and the UK-FA@Au@MSNs with NIR group were the best, but the control group had almost no variety. In order to further study the condition of thrombus residual, the treated blood vessels were stained with hematoxylin and eosin (H&E), and the results (Figure 5B) further confirmed the effection of the UK-FA@Au@MSNs treatment.

2.5. Biosafety Assessment In Vivo

H&E staining images of major organs were exhibited as no signs of histopathological abnormalities such as inflammation or tissue damage after administration of UK-FA@Au@MSNs. Besides, in order to detect changes in coagulation function in animals, we examined the time of Prothrombin time(PT), activated partial thromboplastin time(APTT), thrombin time(TT) and the content of fibrinogen(FIB). As shown in Table 1 (Figure 6). The results showed the time was significantly prolonged (PT>3 s, TT>3 s, APTT>10 s vs control group) and the content of fibrinogen(FIB) (Normal range:2~4 g/L) decreased after treatment with

UK-FA@Au@MSNs ＆NIR. After the treatment with drugs, the thrombus activity decreased and the therapeutic effect was achieved, but there were no caused side effects such as bleeding, which suggested that the system has certain biosafety in the body (Figure 6).

Conclusions

The present work describes a simple synthesis of nanoparticles loaded with UK that exhibits a good thrombolytic effect under NIR. In order to study the thrombolysis of UK-FA@Au@MSNs, in vitro and in vivo experiments were performed, and the residual state of rabbit femoral vein thrombosis was observed in combination with B-ultrasound imaging and H&E staining of intravascular thrombus, and the cytotoxicity of human umbilical vein endothelial cells was evaluated. At the same time, the release amount of lactate dehydrogenase (LDH), Prostacyclin (PGI_2), Nitric Oxide(NO) of cells was evaluated by ELISA. The results showed that the drugs synthesized at the same dose in the vitro experiment showed the darkest color and the shortest thrombolysis time under the NIR. In the vivo experiment, B-ultrasonography was observed less thrombus residue. Because the synthetic material containing fatty acids could protect high-density lipoprotein and reduce the occurrence of atherosclerosis, so it had a certain proliferative effect on vascular endothelial cells. These results openned up new prospects for clinically developed controllable and accurate delivery of thrombolytic agents for thrombus sites in the body.

Experimental Section

Materials:

Cetyltrimethyl ammonium bromide (CTAB), tetraethylorthosilicate (TEOS), etrachloroauricacid ($HAuCl_4 \cdot 4H_2O$), formaldehyde solution (37.0 wt%), sodium hydroxide (96.0 wt%), absolute anhydrous ethanol (99.7 wt%), fluorescence isothiocyanate (FITC), UK (5-6) $\times 10^4$ IU/mg), thrombin, Lauric acid (96.0 wt%) and formalin solution were purchased from Macklin Co. (Shanghai, China). Stearic acid (96.0 wt%) was purchased from Sigma Co. (St.Louis, MO, USA). Methanol (99.5 wt%), N, N-dimethylformamide (DMF, ≥99.5 wt%) XiLong Chemical (Guangdong, China). Presto blue cell viability reagent were purchased from ThermoFisher Co. (USA). RPMI 1640 medium, fetal bovine serum, and penicillin streptomycin were

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supplied by Gibco CO (USA). Nitric Oxide(NO), Prostacyclin (PGI_2), lactate dehydrogenase (LDH) ELISA Kit was purchased from Jianglai biological Co. (Shanghai, China).

Preparation of Au@MSNs

All glasswares were cleaned in a bath of prepared aqua regia (HCl:HNO_3, 3:1 by volume) and rinsed thoroughly in water prior to use，0.4 g of CTAB was dissolved in a solution containing 240 mL of distilled water and 4.8 mL of 0.5 M NaOH. After stirring the reaction for 15 minutes in an environment of 80℃, 8 mL of 37% acetaldehyde was added, and 3.2 mL of 0.029 M $HAuCl_4$ was stirred for 10 minutes. A solution containing 2 g TEOS and 4 g ethanol was added. The products were obtained by centrifugation at 11000 RCF for 20 min after reacting for 90 min, followed by washed four times with water and once with absolute ethanol, and then dried at 55 ℃ overnight. Removal of the surfactant template was achieved by calcination in air from room temperature to 550 ℃ for 6 hrs at the rate of 1 ℃ min^{-1}.

Preparation of UK-FA@Au@MSNs particles

2 mg UK was dissolved in 10 mL methanol, then 6 mL PCM was added to the above solution. 15 mg Au@MSNs was added to 30 mL DMF, sonicated for 20 min to dissolve the particles in the solvent, then added the above solution dropwise, the temperature rose from room temperature to 80 ℃ and stirred for 90 min. Centrifuge at 4 ℃, 11000 rpm for 15 minutes, washed off the excessed UK. The UK-loaded products were freeze-dried at -65 ℃.

Characterization

In the previous step, we synthesized a silicon-coated gold core-shell nanosp here (Au@MSNs) 22 by a classical one-step method. The size and feature o f the synthesized nanoparticles were measured by.scanning electron microscopy (SEM, Zeiss/Sigma 300). In order to analyze the pore size and porosity of the

synthesized Au@MSNs material, the specific surface area was measured by the gas adsorption Brunauer-Emmett-Teller（BET）method. Next, a binary eutectic mixture of lauric acid (LA) and (SA) as a plugging material for mesoporous si licon was added. A transmission electron microscope (TEM, JEM-2010 type; Ja pan Electronics Co.) scan was used to further confirm whether the UK-FA@U K-FA@Au@MSNs was successfully synthesized. Fourier transform infrared spec tra (FTIR, model 6300, Bio-Rad Co. Ltd, USA) were employed to analyze the physical properties of Au@MSNs, UK-FA@Au@MSNs, respectively.

Ultrasonic diagnostic machine, Phillip (USA) NIR laser (808 nm) was supplied by Hi-Tech Optoelectronics Co.Ltd, China. UK and the cytocompatability of m aterials were measured by enzyme-linked immunosorbent assay (SpectraMax M5 type, Tianjin Science and Technology Development Co.). H&E staining was ob served by fluorescence microscope.

Fabrication of PCM nanoparticles

The PCM nanoparticles were fabricated derived from nanoprecipitation via a method. Briefly, 228 mg lauric acid and 72 mg stearic acid (76%：24% by weight) were first dissolved in methanol at a concentration of 10 mg mL^{-1}. For the fabrication of PCM nanoparticles made of single-component fatty acids, the concentration of lauric acid or stearic acid was fixed at 10 mg mL^{-1}. The preparation process involved pouring the mixture samples into 100mL beakers separately, and finally using a magnetic stirrer at 400 rpm to stir the mixture at 70°C for 45 min. Added 30 mL of methanol, then store the PCM at 4 °C.

Drug encapsulation efficiency and drug loading efficiency

The amount of UK-loaded in UK-FA@Au@MSNs was tested by an enzyme-linked immunosorbent assay (ELISA). The protein content in the supernatant was detected after high-speed centrifugation (11,000*g). Finally, the protein content could be measured from the supernatant. The amount of UK-loaded was calculated from total

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UK by subtracting the measured. The drug encapsulation efficiency was defined as the ratio of the drug amount in the ultimately product to the total drug amount added in the process. The drug loading efficiency was defined as the ratio of the drug amount in the final product to the NP amount, which could be determined by lyophilizing the purified NPs. The experiments were performed three times independently for each sample.

Releasing rate of UK-FA@UK-FA@Au@MSNs

To study the feasibility of the drug, we studied the releasing rate of the designed drug delivery system under near-infrared light. The drug delivery system was divided into experimental group and control group. The experimental group was treated with near-infrared irradiation (near-infrared intensity 0.66 W/cm^2), and the control group was placed in an incubator at 37 °C. At the fifth and tenth minutes after the start of the experiment, samples were taken separately, and the concentration of urokinase was measured by enzyme-linked immunosorbent assay. It was then measured every ten minutes, and the data was recorded and statistically analyzed.

Cell Culture and Cytotoxicity Evaluation

Human umbilical vein endothelial cell (HUVEC) was seeded at 2 ×10^3 cells for CCK8 and 5×10^3 for ELISA per well in a 96-well microtiter plate and cultured with RPMI 1640 medium containing 10% fetal bovine serum (FBS, Gibco), 100 U mL^{-1} of penicillin and 100 μg mL^{-1} streptomycin at 37°C under 5% CO_2 atmosphere. The culture medium was replaced with fresh media containing UK-FA@Au@MSNs at a concentration of 100 μg mL^{-1}. The cells were further incubated at 37°C for another 1d, 2d, 3 d, 4d for CCK8, respectively. Then, the cells were washed and incubated with PrestoBlue (PB) reagent. The absorbance was recorded at 450 nm after 2 hrs incubation of endothelial cells with PB reagent. Another part of the cell cultured 24 hours to measure the release of lactate dehydrogenase (LDH), Prostacyclin (PGI_2) and NO by ELISA. The cell viability and Leakage were expressed as a percentage relative to the cells untreated with plant compounds.

Blood collection and clot formation

The blood clot was prepared via adult healthy rabbit. 5 mL blood was collected from healthy adult rabbit ear vein extraction then divided into several tubes. Which

contained 40 U/mL thrombin solution, the test tube was placed at 4°C for 3 days to obtain stable plaque.

Thrombolysis Assessment In Vitro

The experiment was divided into four groups, which were the control group(I), the NIR group (II), the UK group (III), and the UK-FA@UK-FA@Au@MSNs + NIR group(Ⅳ). The prepared blood clot was placed into a transparent bottle containing 5 mL saline, respectively. The Holmstrom method was used as an effective means of checking the clot lysis activity and simulating the blood flow environment(Tadayon Ateke et al； 2015)[17]. The blood clots were photographed at setting time. The NIR groups were treated via NIR laser (0.66 W/cm$^{2)}$ for the indicated time points.

Thrombolysis Assessment In Vivo

All animal procedures were performed according to the protocol approved by the Institutional Animal Care and Use Committee of China. 10 % chloral hydrate, 2 mL/kg rabbit ear vein injection anesthesia, disinfection, along the proximal left leg along the femoral vein, to make a 4 cm long oblique skin incision, free femoral vein about 2 cm. Near heart end clamped with a blood vessel clip, and the distal vessel wall was clamped 10 times with a smooth forcep. The femoral vein was parallel inserted into a 1 mL syringe at a distance of about 1 cm from the distal end of the vessel clamp, and 0.1 mL of thrombin was injected (final concentration 150 U⁄mL), quickly withdrawed the needle and pressed the needle hole for a few minutes. The blood vessel clamp was kept for 2 hrs, and saline was added dropwise to the tissue from time to time to keep it moist. After 2 hrs, the vessel clamps were slowly removed and the muscles and skin were layered suture. The limbs of the control side were not treated. Twelve rabbits were divided randomly into four groups, respectively treated with untreated, UK(2mg in one rabbit), NIR and UK-FA@Au@MSNs (the amount of drug injected was 8mg in one rabbit). Inner thigh of rabbit thigh was irradiated by NIR laser(Power is 2W / cm^2) for 30 min; the distance between laser source and skin was 3.5cm； laser spot size was a circle with a radius of 0.5 cm (about 0.78 cm2). The penetration depth of the light was 0.8cm.) via intravenous injection after emerging blood clots. The dissolution of the thrombus was observed in the Day 0,1,2,3 with B-ultrasound. Referring to the scale in the B-ultrasound images, observed whether the initial size of the thrombus in the four groups were approximately the same. After the rabbit was sacrificed, the major organs and the vascular tissue at the

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thrombus were taken for H&E staining. Three stained sections were chosen randomly to image and analysis in each slice.

Declarations

Availability of data and material:

The authors declare that [the/all other] data supporting the findings of this study are available within the article [and its supplementary information files]

Consent for publication:

Not applicable

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Ethical approval and consent to participate:

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Disclosure statement

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References

1. Kyrle, P. A.; Eichinger, S., Deep vein thrombosis. *Lancet* **2005,** *365* (9465), 1163-74.

2. Heit, J. A., The epidemiology of venous thromboembolism in the community: implications for prevention and management. *J Thromb Thrombolysis* **2006,** *21* (1), 23-9.

3. Kahn, S. R.; Partsch, H.; Vedantham, S.; Prandoni, P.; Kearon, C., Definition of post-thrombotic syndrome of the leg for use in clinical investigations: a recommendation for standardization.[J] .J. Thromb. Haemost., 2009, 7: 879-83.

4. Jin, H. J.; Zhang, H.; Sun, M. L.; Zhang, B. G.; Zhang, J. W., Urokinase-coated chitosan nanoparticles for thrombolytic therapy: preparation and pharmacodynamics in vivo. *J Thromb Thrombolysis* **2013,** *36* (4), 458-68.

5. Schweizer, J.; Kirch, W.; Koch, R.; Elix, H.; Hellner, G.; Forkmann, L.; Graf, A., Short- and long-term results after thrombolytic treatment of deep venous thrombosis. *Journal of the American College of Cardiology* **2000,** *36* (4), 1336-1343.

6. Guo, R.; Tian, Y.; Wang, Y.; Yang, W., Near-Infrared Laser-Triggered Nitric Oxide Nanogenerators for the Reversal of Multidrug Resistance in Cancer. *Advanced Functional Materials* **2017,** *27* (13).

View Article Online
DOI: 10.1039/C9TB01851D

7. Peng, H.; Tang, J.; Zheng, R.; Guo, G.; Dong, A.; Wang, Y.; Yang, W., Nuclear-Targeted Multifunctional Magnetic Nanoparticles for Photothermal Therapy. *Adv Healthc Mater* **2017,** *6* (7).

8. Chen, C.-W.; Syu, W.-J.; Huang, T.-C.; Lee, Y.-C.; Hsiao, J.-K.; Huang, K.-Y.; Yu, H.-P.; Liao, M.-Y.; Lai, P.-S., Encapsulation of Au/Fe3O4 nanoparticles into a polymer nanoarchitecture with combined near infrared-triggered chemo-photothermal therapy based on intracellular secondary protein understanding. *Journal of Materials Chemistry B* **2017,** *5* (29), 5774-5782.

9. Piras, A. M.; Chiellini, F.; Fiumi, C.; Bartoli, C.; Chiellini, E.; Fiorentino, B.; Farina, C., A new biocompatible nanoparticle delivery system for the release of fibrinolytic drugs. *Int J Pharm* **2008,** *357* (1-2), 260-71.

10. Zhang, N.; Li, C.; Zhou, D.; Ding, C.; Jin, Y.; Tian, Q.; Meng, X.; Pu, K.; Zhu, Y., Cyclic RGD functionalized liposomes encapsulating urokinase for thrombolysis. *Acta Biomater* **2018,** *70*, 227-236.

11. Huang, X. H.; Jain, P. K.; El-Sayed, I. H.; El-Sayed, M. A., Plasmonic photothermal therapy (PPTT) using gold nanoparticles. *Laser Med Sci* **2008,** *23* (3), 217-228.

12. Du, B.; Gu, X.; Zhao, W.; Liu, Z.; Li, D.; Wang, E.; Wang, J., Hybrid of gold nanostar and indocyanine green for targeted imaging-guided diagnosis and phototherapy using low-density laser irradiation. *Journal of Materials Chemistry B* **2016,** *4* (35), 5842-5849.

View Article Online
DOI: 10.1039/C9TB01851D

13. Zhu, H.; Zhang, P.; Meng, Z. N.; Li, M., Thermal Characterization of Lauric-Stearic Acid/Expanded Graphite Eutectic Mixture as Phase Change Materials. *J Nanosci Nanotechno* **2015,** *15* (4), 3288-3294.

14. Mensink, R. P.; Zock, P. L.; Kester, A. D. M.; Katan, M. B., Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am J Clin Nutr* **2003,** *77* (5), 1146-1155.

15. Chen, J.; Zhang, R.; Han, L.; Tu, B.; Zhao, D., One-pot synthesis of thermally stable gold@mesoporous silica core-shell nanospheres with catalytic activity. *Nano Research* **2013,** *6* (12), 871-879.

16. Zhu, C. L.; Huo, D.; Chen, Q. S.; Xue, J. J.; Shen, S.; Xia, Y. N., A Eutectic Mixture of Natural Fatty Acids Can Serve as the Gating Material for Near-Infrared-Triggered Drug Release. *Adv Mater* **2017,** *29* (40).

17. Tadayon Ateke,Jamshidi Reza,Esmaeili Akbar,Delivery of tissue plasminogen activator and streptokinase magnetic nanoparticles to target vascular diseases.[J] .Int J Pharm, 2015, 495: 428-438.

18. Jin, R. C.; Loscalzo, J., Vascular Nitric Oxide: Formation and Function. *J Blood Med* **2010,** *2010* (1), 147-162.

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DOI: 10.1039/C9TB01851D

19. Handa, H.; Major, T. C.; Brisbois, E. J.; Amoako, K. A.; Meyerhoff, M. E.; Bartlett, R. H., Hemocompatibility Comparison of Biomedical Grade Polymers Using Rabbit Thrombogenicity Model for Preparing Nonthrombogenic Nitric Oxide Releasing Surfaces. *J Mater Chem B* **2014,** *2* (8), 1059-1067.

20. Brisbois, E. J.; Davis, R. P.; Jones, A. M.; Major, T. C.; Bartlett, R. H.; Meyerhoff, M. E.; Handa, H., Reduction in thrombosis and bacterial adhesion with 7 day implantation of S-nitroso-N-acetylpenicillamine (SNAP)-doped Elast-eon E2As catheters in sheep. *Journal of Materials Chemistry B* **2015,** *3* (8), 1639-1645.

21. Jones, G.; Hurley, J. V., The Effect of Prostacyclin on the Adhesion of Leukocytes to Injured Vascular Endothelium. *J Pathol* **1984,** *142* (1), 51-59.

22. Dusting, G. J.; Moncada, S.; Vane, J. R., Prostacyclin (PGX) is the endogenous metabolite responsible for relaxation of coronary arteries induced by arachindonic acid. *Prostaglandins* **1977,** *13* (1), 3-15.

23. Higgs, E. A.; Higgs, G. A.; Moncada, S., .; Vane, J. R., Prostacyclin (PGI2) inhibits the formation of platelet thrombi in arterioles and venules of the hamster cheek pouch. *Br J Pharmacol* **1997,** *63* (3), 535-539.

Figure

Figure 1:Depict aslo system provides a localized hyperthermia for DVT therapy

Figure 2A：Scanning electron microscope (SEM) images showed the morphology of Au@MSNs

Figure 2B：TEM image of synthesized Au@MSNs.

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Figure 2C：The measured Brunauer-Emmett-Teller (BET) surface area

Figure 2D The dynamic light scattering (DLS) results of Au@MSNs

Figure 2E：Urokinase release rate. The red curve represents the urokinase release rate of the drug system under the irradiation of near-infrared light with an intensity of 0.66 W / cm2; the black curve represents the urokinase release rate of the drug system under a constant temperature environment of 37° C.

Figure 2F：The relationship between heating rate and concentration under the NIR

Figure 3A：The clot lysis in the four groups in the absence of flow

Figure 3B：The clot lysis in the four groups in the presence of flow

Figure 4A：Relative lactate dehydrogenase (LDH) release

Figure 4B：Relative Nitric Oxide(NO) release

Figure 4C：Relative Prostacyclin (PGI_2) release

Figure 4D：HUVECs were treated with UK-FA @ UK-FA @ Au @ MSNs concentrations of 0, 1, 10, and 100 ug / ml, respectively, and cell viability was measured by CCK8 experiments.

Figure 5A：The thrombus in the treatment group was dissolved compare with untreated

Figure 5B：The treated blood vessels were stained with hematoxylin and eosin (H&E)

Figure 6: H&E staining images of major organs and examined the time of prothrombin time(PT), activated partial thromboplastin time(APTT), thrombin time(TT) and the content of fibrinogen(FIB)

Figure S1. TEM image of synthesized Au@MSNs.

Figure S2. FTIR spectra of Au@MSNs and UK-FA@Au@MSNs.

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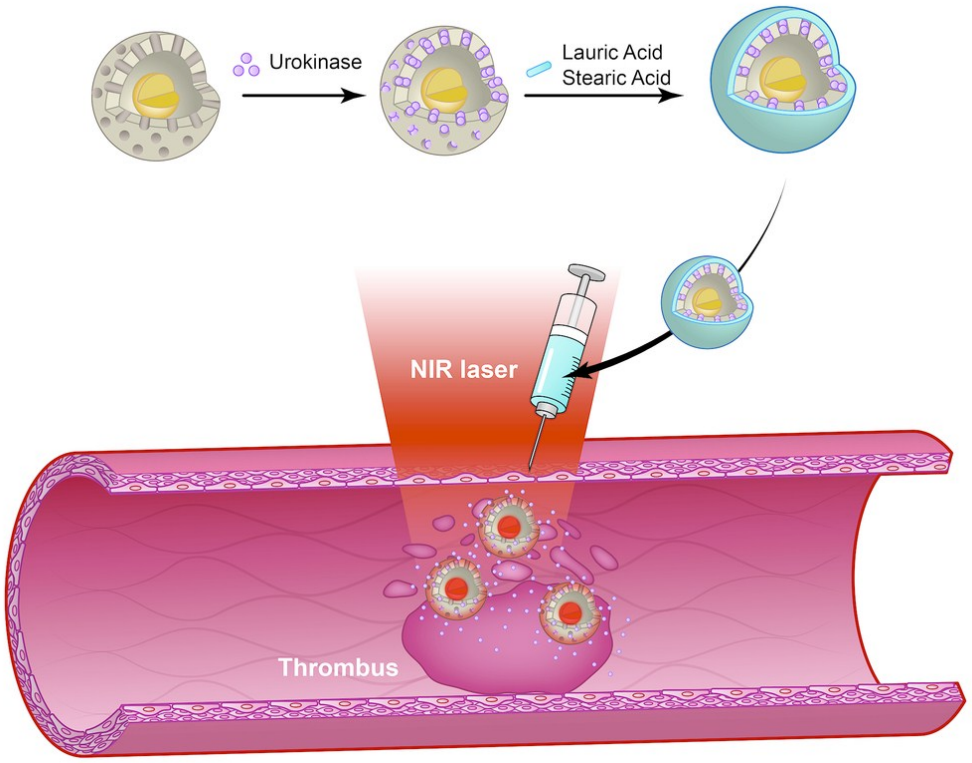

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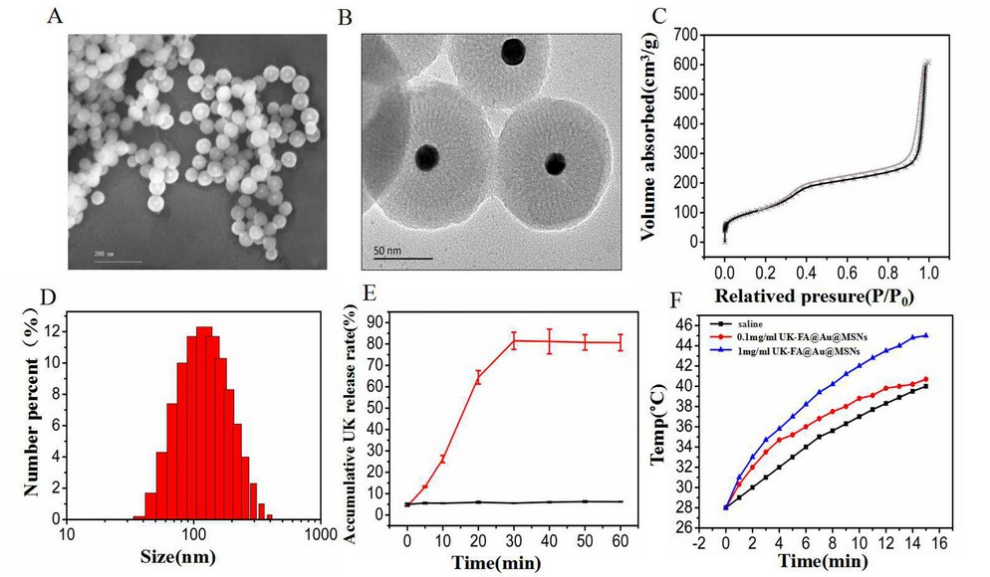

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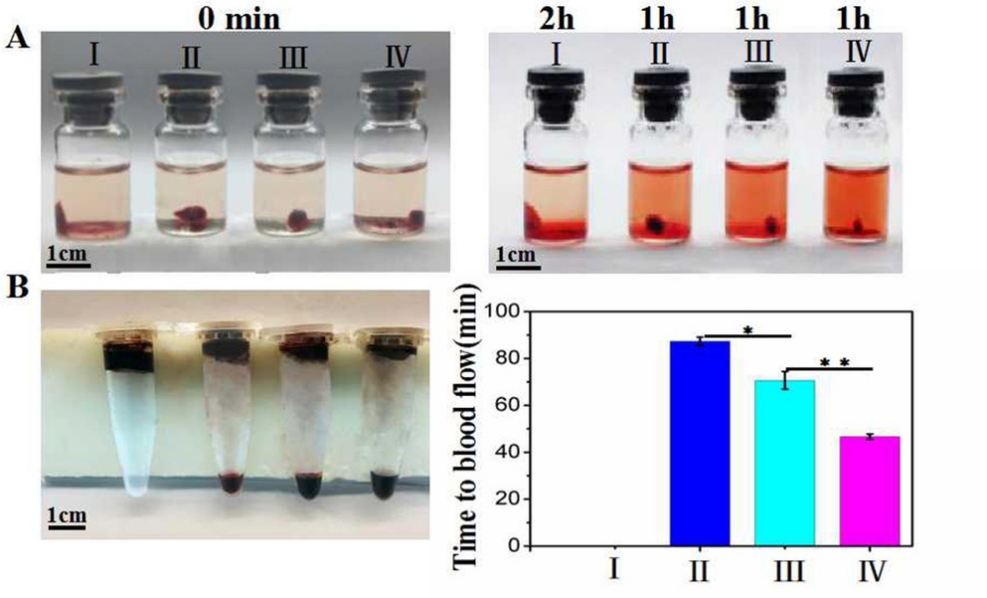

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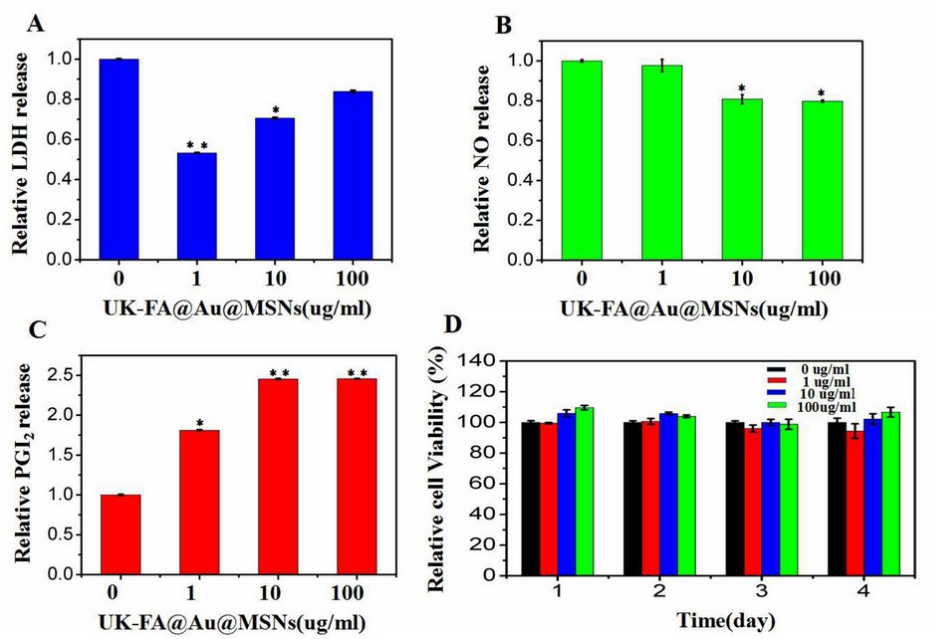

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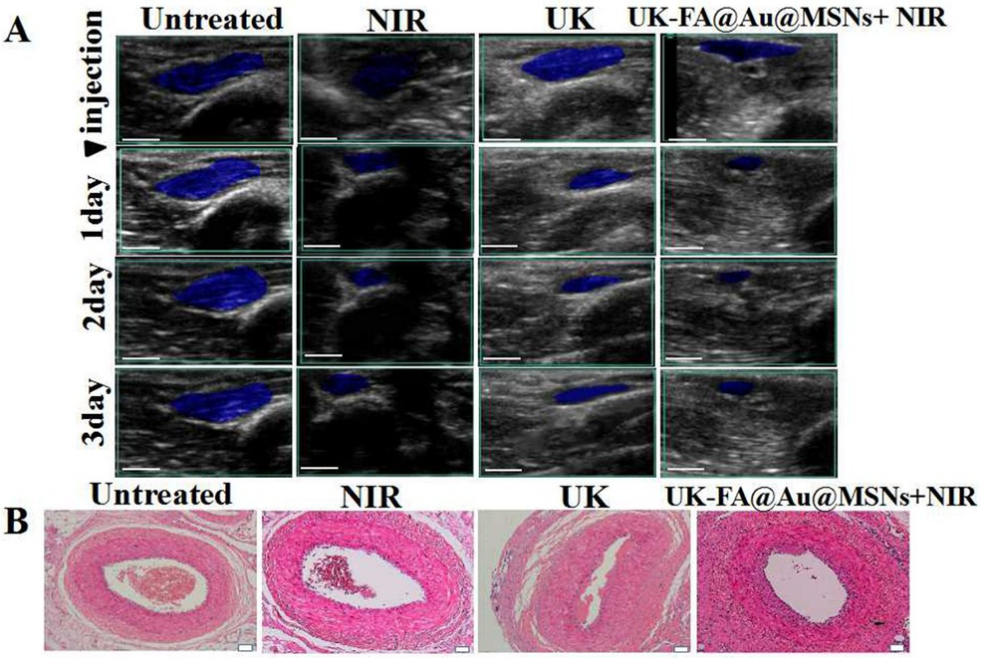

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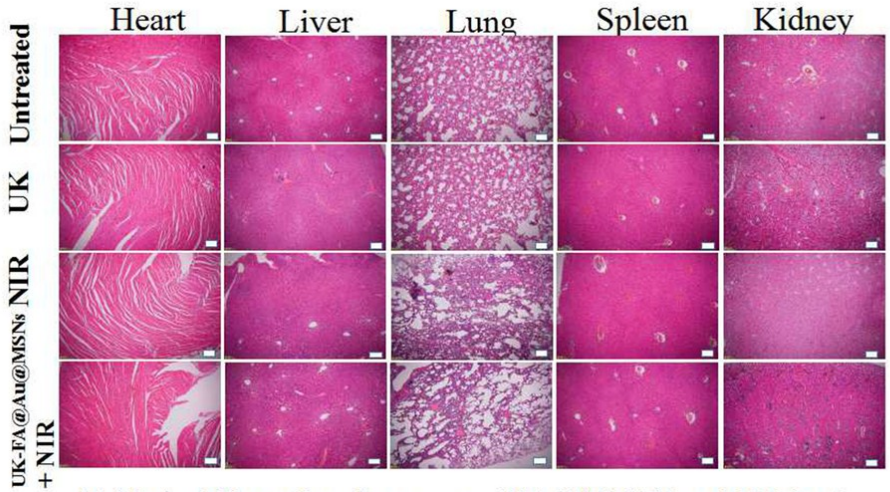

Table 1 Effect of each group on PT, APTT, TT and FIB in vivo

	Untreated	UK		NIR		UK-FA@Au@MSNs + NIR	
		treatment		treatment		treatment	
		Before	after	Before	after	Before	after
PT(S)	7.5 ±1.5	7.4 ±0.2	7.8±0.2	6.75±0.65	7.5±0.4	7.83±0.24	11.65±0.25
APPT(S)	17.2 ±0.3	18.2 ±1.1	22.1 ±0.3	17.85±1.05	21.5±1.7	21.2±0.73	21.95±0.55
TT(S)	13.7 ±2.6	13.65±0.25	20.35±3.15	15.75±4.05	16.25±2.55	14.77±1.51	22.55±1.15
FIB(g/L)	5.05 ±0.28	5.33±0.01	3.75±0.94	4.81±1.16	3.86±1.85	3.67±0.17	3.57±0.29

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