Supporting Information

Human VEGF magnetic molecularly imprinted polymer for drug-free anti-angiogenesis and photothermal therapy of tumors

Mengzhao Wen[a], Haizhu Shi[a], Yu Wan[a], Jiateng Wu[a], Xiao Tian[a], Qian Chen[a], Ming-Yu Wu*[a], Shun Feng*[a]

[a]*Sichuan Engineering Research Center for Biomimetic Synthesis of Natural Drugs, School of Life Science and Engineering, Southwest Jiaotong University, Chengdu 610031, China. E-mail: wumy1050hx@swjtu.edu.cn, fengshunxd@hotmail.com.*

Table of Contents

1. Experimental section

1.1 Reagents and Materials

9-Fluorenylmethoxycarbonyl (Fmoc) amino acids, N, N, N′, N′-tetramethyl-O-(1H-benzotriazol-1-yl) uronium hexafluorophosphate (HBTU), 1-hydroxybenzotriazole monohydrate (HOBT), N, N-diisopropylethylamine (DIPEA), triisopropylsilane (TIS), piperidine, N, N, N', N'-tetramethylethylenediamine (TEMED), Tris-HCl (pH=6.8/8.8), glycine (Gly), 30% acrylamide, ammonium persulfate, ECL chemiluminescence kit and anhydrous ether were purchased from Aladdin Biochemical Reagent Co., Ltd. (Shanghai, China). Anhydrous dimethylformamide (DMF), anhydrous dichloromethane (DCM), trifluoroacetic acid (TFA) and Wang resin were purchased from J&K Science Co., Ltd. (Beijing, China). Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), γ-methacryloxypropyl trimethoxy silane (MPS), 1,6-hexamethylenediamine, Tween 20, Coomassie brilliant blue, fluorescein isothiocyanate isomer I (FITC) was purchased from Adamas Reagent Co., Ltd. (Shanghai, China). VEGFA polyclonal antibody rabbit polyclonal, HRP-conjugated affinipure goat anti-rabbit IgG(H+L) was purchased from Proteintech Reagent Co., Ltd., (Wuhan, China). Pageruler pre-staining protein markers were purchased from Thermofisher Science Co., Ltd. (Shanghai, China). Phosphate buffer solution for cell culture (1×PBS), Dulbecco phosphate buffer saline (DPBS), parenzyme cell digestion solution (containing 0.25% trypase and 0.02% EDTA), Dulbecco modified eagle medium (DMEM, containing 4.5 mg/mL glucose, 80 U/mL penicillin and 0.08 mg/mL streptomycin), fetal bovine serum (FBS) and calf serum were purchased from Gibco (Life Technologies, Australia). Annexin V-Alexa Fluor 647-PI kit, BCA kit, and Calcein AM kit were purchased from Beijing Solarbio Technology Co., LTD., China. CCK-8 kit were purchased from Biosharp. Bevacizumab was purchased from MCE. The angiogenesis assay kit was purchased from Corning. High-performance liquid chromatography (HPLC) solvents were purchased from Fisher Scientific (US). All chemical reagents were of analytical grade unless otherwise stated.

1.2 Instruments.

The morphology of the nanoparticles was observed by Transmission electron microscopy (TEM, JEOL-2100, JEOL Ltd., Japan). Western blot was performed on a gel imaging device (Bio-Rad Power PacBasic California, USA). The VEGF epitope peptides were isolated and purified by HPLC (Waters 1500, MA, USA); and identified by chromatography-mass spectrometry (Xevo G2-S Tof, Waltham, MA, USA). The fluorescence of cells was detected by a flow cytometer (BD Accuri C6, New Jersey, USA). The particle size and the ζ-potential were measured on Nano-ZS ZEN3600 (Malvern Instruments, UK) at 25°C. The 808 nm NIR laser was applied to carry out the photothermal therapy (PTT) study (LWIRL808-0-15W-F, Beijing Laserwave Optoelectronic Technology Co., Ltd., China). The temperature variation was monitored by a FLIR camera (FLIR T420, USA). Cell viability was detected by the Varioskan flash enzyme labeling instrument (Thermo, USA). Apoptosis morphology of cells was observed by an inverted fluorescence microscope (CKX41, Olympus, Japan).

1.3 Synthesis of hVEGF Epitope Peptide

The epitope peptide of hVEGF (IKPHQGQHI) was synthesized by Fmoc solid-phase peptide synthesis strategy.[1] Wang resin was used as the initial material for the synthesis. The resin (0.5 g) was swollen with DMF for about 2 h and alternately washed with DMF and DCM three times. Then, Fmoc-amino acid (3 eq), HATU (3 eq), and HOBT (3 eq) dissolved in DMF (containing DIPEA (6 eq)) were added into the resin, the coupling step was continued for 2 h. The unreacted amino groups were quenched by methanol. Then the Fmoc-protected groups were deprotected with 20% piperidine/DMF (v/v) twice. The deprotection and coupling cycles were repeated until the last amino acid was successfully coupled to the resin. The cleavage of the peptide from the resins was achieved using the cleavage cocktail (95%TFA, 2.5%H_2O, 2.5%TIS) for 2 h. After that, the cleavage solution was filtered, then the peptides were precipitated with cold diethyl ether at -20°C overnight. Then, the product was lyophilized and stored at -20°C. Finally, the purity of the peptide was determined by

high-performance liquid chromatography with a C18 reversed-phase column using a linear gradient from 5 to 95% of acetonitrile (0.1% TFA) to run for 40 min. The molecular weights of the peptides were measured by electrospray ionization mass spectrometry.

1.4 Preparation of Fe_3O_4-NH_2 Nanoparticles

Fe_3O_4-NH_2 nanoparticles (NPs) were prepared by the solvothermal method.[2] Briefly, $FeCl_3$ (1 g) was added to glycol (30 mL) and dissolved under ultrasonic. Then, anhydrous sodium acetate (1 g) and 1, 6-hexanediamine (10 mL) were added under mechanical stirring for 45 min. The mixed solution gradually changed from brown to transparent reddish brown with the increased stirring time. Finally, the mixture was transferred to a 100 mL polytetrafluoroethylene reactor and reacted at 205°C for 6 h. The magnetite NPs were then alternately washed with water and ethanol 3 times to effectively remove solvents and unreacted substances and then dried under 50°C.

1.5 Preparation of Molecularly Printed Polymer NPs

Molecularly printed polymer (MIP) NPs were synthesized by sol-gel method and epitope blotting. With epitope peptide of hVEGF as a template, MPS as a functional monomer and cross-linking agent, and Fe_3O_4-NH_2 as the core. In short, the synthetic Fe_3O_4-NH_2 (20 mg) and MPS (2 μL), 0.1% ammonia (10 μL) was dispersed in Tween-20 solution (6 mL), reacted at room temperature for 12 h, and the intermediate was obtained by magnetic separation. Then the modified Fe_3O_4-NH_2, hVEGF peptides (2 mL, 1 mg/mL), MPS (0.5 μL), and 0.1% ammonia (40 μL) were dispersed in Tween-20 solution (6 mL) and reacted at room temperature for 12 h. The final product was obtained by magnetic separation. Then the template molecules were eluted under the action of eluent solution (methanol: glacial acetic acid = 9:1). Finally, the black powder was obtained by freeze-drying, which was recorded as hVEGF-MIP.

Meanwhile, non-imprinted polymer (NIP) NPs were prepared in parallel, except no template molecule was added.

1.6 Adsorption Experiment of hVEGF-MIP and NIP

The isothermal adsorption curves were tested by immersing hVEGF-MIP or NIP NPs in epitope peptide solutions with concentrations ranging from 0 - 150 μg/mL, respectively. After 2 h absorption, the residues of peptide in supernatants obtained by magnetic separation were determined by UV-Vis spectrometer at 264 nm.

The adsorption kinetics studies were carried out at different time points (0 - 240 min). Unless further declaration, all tests were repeated three times. Adsorption capacity Q (mg/g) of hVEGF-MIP or NIP towards the epitope peptide of hVEGF was calculated according to Eq. 1. And the binding constant and the maximum apparent adsorption capacity of hVEGF-MIP were calculated according to Eq. 2. The adsorption kinetic mechanism was further analyzed by fitting equations of the pseudo-first and pseudo-second-order kinetic models (Eq. 3 and Eq. 4), respectively.

$$Q = \frac{(C_0 - C_e) \times \mathrm{V}}{m} \quad (1)$$

$$\frac{Q_e}{C} = \frac{Q_{max} - Q_e}{K_d} \quad (2)$$

$$\ln (Q_e - Q_t) = lnQ_e - K_1 t \quad (3)$$

$$\frac{t}{Q_t} = \frac{1}{K_2 Q_e^2} - \frac{t}{Q_e} \quad (4)$$

where C_0 and C_t are the initial and residual concentration of the solution at different time points (μg/mL). V is the volume of the epitope of hVEGF solution (mL), and m is the weight of the adsorbents (mg). Q_e (mg/g) and C_e (μg/mL) are the equilibrium amount and the concentration of epitope of hVEGF, respectively. Q_{max} is the apparent maximum binding capacity of the hVEGF-MIP (mg/g). k_d is dissociation constant (L/mg). Qt is the amount of epitope of hVEGF adsorbed at the time t (mg/g), respectively. k_1 (1/min) and k_2 (g/(mg·min)) are the rate constant of the pseudo-first-order and pseudo-second-order adsorption, respectively.

1.7 Selective and Specific Experiments of hVEGF-MIP

In order to simulate the complex action environment of MIP in vivo, cell lysate was used as the incubation mixed solution. In brief, human cervical cancer (HeLa), mouse breast cancer (4T1) and mouse melanoma cells (B16) cells were separately seeded at

2×10^5 per well into 6-well cell culture plates and cultured at 48 h at 37ºC, 5% CO_2. After being cultured for 48 h, the cell culture supernatant was removed, and the HeLa, 4T1 and B16 cells were washed with pre-cooled PBS (pH 7.4) three times to remove unbound nanoparticles. After that, cell lysis buffer containing protease inhibitor and phosphatase inhibitor was added to the cells and the cells were lysed on ice for 30 min. Cells were scraped with clean cell scrapes and transferred into centrifugal tubes with a pipette. The lysate mixture was centrifuged at -4°C at 12,000 rpm for 5 min, and the supernatant was collected for further use.

Then a certain amount of hVEGF-MIP was incubated with lysate supernatant for 4 h. After incubation, the supernatant and hVEGF-MIP were collected by magnetic separation. hVEGF-MIP was washed with a certain concentration of sodium dodecyl sulfate solution, and the eluent was collected and used. Then protein concentration in the lysate mixture, supernatant and eluent was quantified using a BCA protein quantitation assay. After that, protein (20 μg) from the lysate mixture, supernatant and eluent was loaded on sodiumdodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). After running SDS-PAGE, the gel was stained overnight with Coomassie brilliant blue solution. Then the gel was decolorized with a washing solution so the protein band could be clearly observed. Finally, the Bio-Rad GelDoc-XRTM gel imaging system was employed to expose the gel and obtain images.

1.8 Photothermal Properties

Before testing the photothermal properties of hVEGF-MIP, the ultraviolet absorption spectrum of Fe_3O_4-NH_2 NPs was measured by ultraviolet spectrophotometer.

hVEGF-MIP (100 μg) was added into water (0.5 mL) solution and exposed to laser irradiation (808 nm, 2 W/cm^2), and the photothermal heating curves at different time points were measured using an infrared thermometer.

The photothermal cycling performance of hVEGF-MIP was performed by irradiating 200 μg/mL hVEGF-MIP *aq* with 2 W/cm^2 808 nm laser for 15 min. Then the solution was cooled to room temperature, naturally. The procedure was repeated 5 cycles.

1.9 Cytotoxicity Test in *vitro*

The human umbilical vein endothelial cells (HUVEC) were cultured in a high glucose DMEM medium containing 10% FBS under a humidified environment of 37ºC, 5% CO_2 and 95% air. The toxicity of hVEGF-MIP, NIP, and Fe_3O_4-NH_2 NPs on HUVEC were tested using a CCK-8 kit. In brief, HUVEC were seeded at 5000 per well into 96-well cell culture plates and incubated at 37ºC, 5% CO_2. After 24 h incubation, the medium was removed, and fresh medium containing different concentrations of gradient hVEGF-MIP, NIP, and Fe_3O_4-NH_2 was added and further cultured for 24 h. Subsequently, CCK-8 (10 μL) was added to each well. About 30 min later, the absorbance was measured at 450 nm by a microplate reader.

The cell viability was expressed as a percentage of the absorbance of test cells (added with NPs) over that of the control experiment (without the addition of NPs) (both were deducted by the background absorbance), which can be calculated by the following equation:

$$Cell\ viability(\%) = \frac{Abs(test) - Abs(background)}{Abs(control) - Abs(background)} \times 100$$

1.10 Proliferation Inhibition Test in *vitro*

The HeLa, human colon cancer (HCT-116), and human hepatoma (HepG-2) cells were cultured in DMEM medium containing 10% FBS in a CO_2 incubator (37°C, 5% CO_2). The CCK-8 kit was used to test the proliferation inhibition of hVEGF-MIP. In brief, cells were seeded at 5000 per well into 96-well cell culture plates and cultured for 24 h at 37ºC, 5% CO_2. After the medium was removed, fresh medium containing different concentrations of gradient hVEGF-MIP and NIP was added and further cultured for 24 h, 48 h or 72 h. Subsequently, CCK-8 (10 μL) was added to each well. About 30 min later, the absorbance was measured at 450 nm by a microplate reader. Inhibition of cell growth was given by the following equation.

$$Cell\ inhibition(\%) = \frac{OD(control) - OD(trentment)}{OD(control) - OD(background)} \times 100$$

1.11 Cellular Uptake Assay

FITC was modified onto hVEGF-MIP or NIP as follows: hVEGF-MIP or NIP (100 mg) were ultrasonic dispersion in anhydrous ethanol (8 mL), FITC ethanol solution (800 μL 100 μg/mL) was added and stirred for 6 h under dark.[3] After magnetic separation, the products were washed with pure water several times and dried under vacuum at 40°C for 12 h to obtain hVEGF-MIP^{FITC} and NIP^{FITC}, respectively.

HeLa cells were seeded into 24-well cell culture plates (2×10^5 per well) and cultured for 24 h at 37°C, 5% CO_2. After the medium was removed, 500 μL of fresh medium containing 200 μg/mL of hVEGF-MIP^{FITC} or NIP^{FITC} was added and further incubated for 6, 12, 24 or 48 h. The culture medium without NPs was used as a control. The cell uptake efficiency of hVEGF-MIP^{FITC} and NIP^{FITC} were detected by flow cytometry.

Besides, hVEGF-MIP was collected from the cell culture supernatant by magnetic separation technique. Then the particle size and Zeta potential of the collected hVEGF-MIP were investigated and compared with the hVEGF-MIP without adsorbed hVEGF.

1.12 In *vitro* Angiogenesis Assay

The anti-angiogenic effect of hVEGF-MIP was investigated by an angiogenesis experiment in vitro[4]. HeLa cells were seeded into 6-well cell culture plates (2×10^5 per well) and cultured for 24 h at 37°C, 5% CO_2. After the medium was removed, 200 μg/mL hVEGF-MIP or NIP was added to each well. An equal volume DMEM medium without NPs was used as a control. After being cultured for another 48 h, the cell culture supernatant was collected and centrifuged at a rate of 1000 rpm/min for 5 min, which can be kept at 4°C for further use.

HUVEC cells were starved overnight in 0.2% DMEM medium, washed with DPBS twice, and digested. HUVEC cells were diluted to 2×10^5 cells per milliliter with culture supernatant of HeLa cell treated differently as described above.

Finally, the Matrigel dissolved at 4°C was inoculated into 48-well cell culture plates at 150 μL per well, and then solidified at 37°C for 30 min. The diluted HUVEC cells were seeded into above 48-well cell culture plates (300 μL per well) and cultured for 8

h at 37°C, 5% CO_2. Tube formation of HUVECs was observed under an inverted light microscope.

In order to further investigate the specific anti-angiogenic ability of hVEGF-MIP, HeLa cell lysate supernatant and 4T1 cell culture supernatant treated by NIP/hVEGF-MIP were co-cultured with HUVEC, respectively. Subsequent experimental operation is the same as above.

1.13 In *vitro* synergistic therapy experiment

HeLa cells were seeded at 5000 per well into 96-well cell culture plates and cultured for 24 h at 37°C, 5% CO_2. After removing the medium, fresh medium containing different concentrations of gradient hVEGF-MIP or NIP was added and further cultured for 6 h. Then cells were irradiated with or without an 808 nm laser (2 W/cm^2) for 5 min. After 24 h of incubation, CCK-8 assay was performed as described above.

Meantime, the photothermal therapy effect of Fe_3O_4-NH_2 NPs on Hela cells was investigated. HeLa cells were set as Fe_3O_4-NH_2 and Fe_3O_4-NH_2 + NIR group and cultured for 24 h at 37°C, 5% CO_2. After removing the medium, fresh medium containing different concentrations of gradient Fe_3O_4-NH_2 was added and further cultured for 6 h. Then Fe_3O_4-NH_2 + NIR group were irradiated with an 808 nm laser (2 W/cm^2) for 5 min. After 24 h of incubation, CCK-8 assay was performed as described above.

1.14 Western Blot

The cell culture supernatant with different treatments was collected and centrifuged at 12000 rpm/min at -4°C for 5 min. Then protein concentration in the cell culture supernatant was quantified using a BCA protein quantitation assay. Protein (20 μg) from the supernatant was loaded on SDS-PAGE. Polyvinylidene fluoride (PVDF) membrane was used to transfer the gels. Then 5% skim milk in TBST (Tris-buffered saline with 0.1% Tween 20) was used to block the blank. The PVDF membrane was incubated with primary antibodies at 4°C overnight (1:2500 dilution). Then secondary

antibodies were added and incubated with a PVDF membrane for 1 h at room temperature (1:3000 dilution). Unbound antibodies were washed away with TBST. Then the PVDF membrane was incubated with an ECL chemiluminescence solution. Finally, the Bio-Rad GelDoc-XRTM gel imaging system was employed to expose the PVDF membrane and obtain images.

1.15 Detection of apoptosis by Calcein AM/PI staining

HeLa cells were cultured with different concentrations of gradient hVEGF-MIP or NIP for 24 h, and then irradiated with or without an 808 nm laser (2 W/cm^2) for 5 min. After the culture medium was removed, HeLa cells were co-stained with Calcein AM and PI for 30 min. The fluorescence images were investigated using a fluorescence microscope.

1.16 Detection of Apoptosis by Flow Cytometry

HeLa cells were cultured with different concentrations of gradient hVEGF-MIP or NIP for 24 h and then irradiated with or without an 808 nm laser (2 W/cm^2) for 5 min. Then the cells were co-stained with Annexin V-Alexa Fluor 647 and PI, and the apoptosis was detected by flow cytometry.

1.17 In vitro Photothermal Therapy with hVEGF-MIP@DOX

Firstly, hVEGF-MIP (10 mg) was incubated with DOX solution (15 mL; 90 μg/mL) overnight. The hVEGF-MIP@DOX was obtained through magnetic separation, washed with water several times, and dried under the freeze. The drug loading and loading rate were calculated by the following formula.

$$Drug\ loading = \frac{((C_0 - C_t) \times V)}{M}$$

$$Drug\ loading(\%) = \frac{weight\ of\ drugs\ in\ carriers}{carrier\ weight} \times 100$$

where C_0 and C_t are the initial and the residual concentration of the DOX solution at the different time points (μg/mL). V is the volume of the DOX solution (mL), and M is the hVEGF-MIP (mg) weight.

To evaluate the photothermal antitumor effect of hVEGF-MIP@DOX, HeLa cells were cultured with different concentrations of gradient DOX, hVEGF-MIP, and hVEGF-MIP@DOX for 24 h and irradiated with or without an 808 nm laser (2 W/cm^2) for 5 min. The cells viability was detected by the CCK-8 kit as above.

1.18 Serum stability test of hVEGF-MIP

Taking the particle size of hVEGF-MIP as the standard, the stability of hVEGF-MIP was investigated in 10% serum environment. A certain amount of hVEGF-MIP was ultrasonically dispersed in 10% serum solution, stored at 25ºC for 48 h, and part of the solution was taken out at specific time points to measure the particle size of hVEGF-MIP.

1.19 Cytotoxicity test of bevacizumab

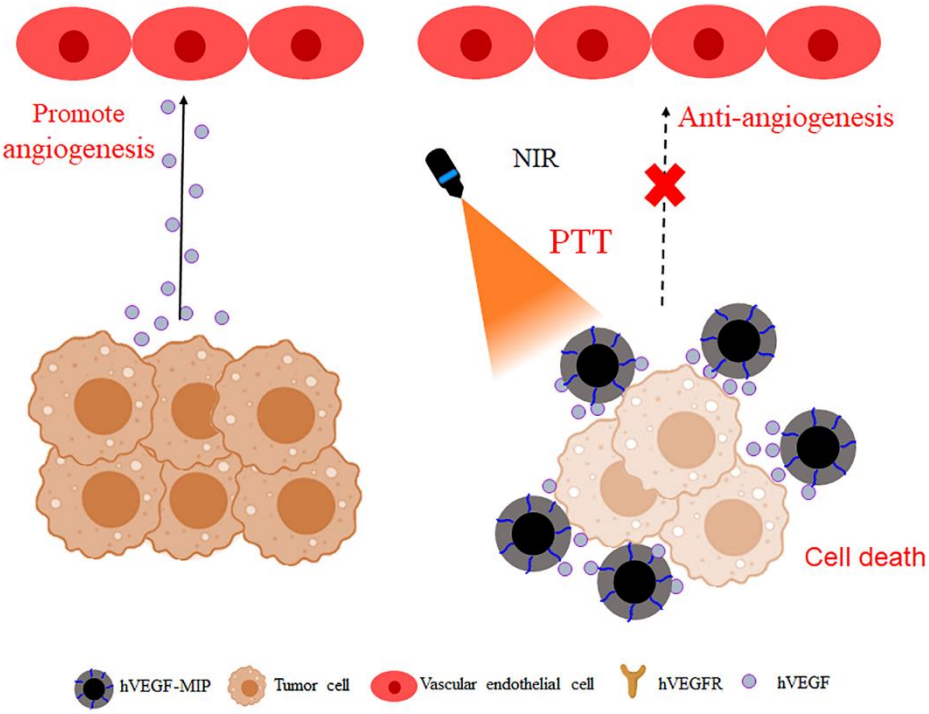
HeLa cells were seeded at 5000 per well into 96-well cell culture plates and cultured for 24 h at 37ºC, 5% CO_2. After removing the medium, fresh medium containing different concentrations of gradient bevacizumab was added and further cultured for 24 h. After 24 h of incubation, CCK-8 assay was performed as described above.

2. Results and discussion

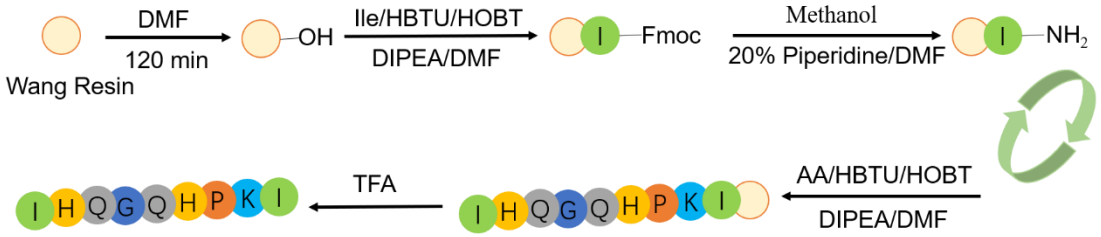
2.1 The Characterization of hVEGF-MIP and NIP.

From the Fourier transform infrared spectroscopy (FT-IR) characterization, the peak at 578 cm^{-1} and 3430 cm^{-1} were attributed to the stretch of Fe-O and -NH_2 from Fe_3O_4 in the three curves. The 1081 cm^{-1} adsorption from both MIP and NIP was attributed to the characteristic peak of Si-O-Si, indicating that the MIP layer was fabricated successfully on the surface of Fe_3O_4. Then, the surface charge of the nanoparticles is evaluated by the Zeta-potential change. Initially, Fe_3O_4-NH_2 is positively charged because the-NH_2 in Fe_3O_4 is positively charged. After the modification of MIP layer, the surface charge of hVEGF-MIP decreased to-22.78 ± 0.2 mV due to the residual negative charge of -OH in the monomer. Compared with hVEGF-MIP, the zeta potential of NIP increases slightly, which may be due to the absence of holes on the surface of NIP.

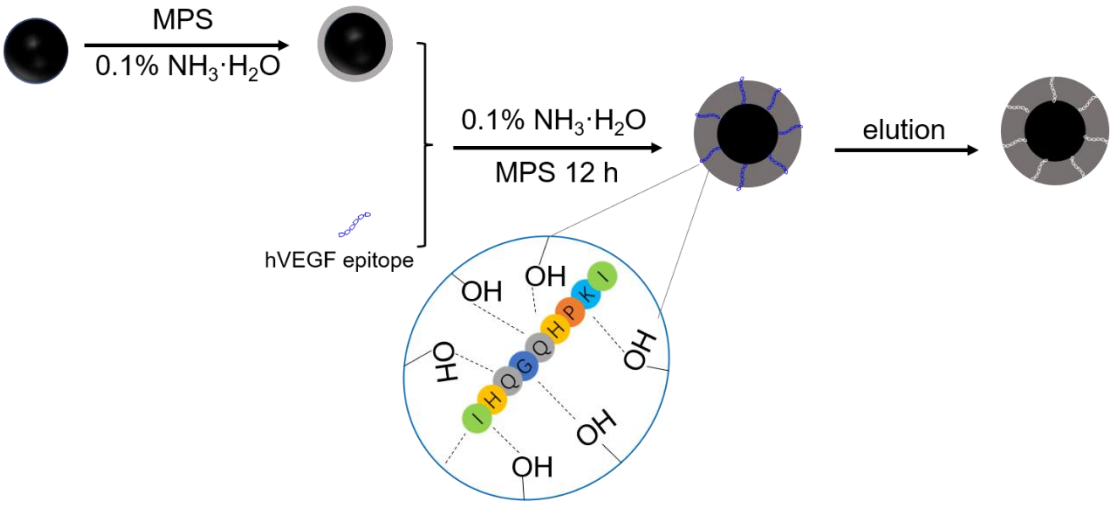
3. Supplementary Data



Scheme. S1 Principle of hVEGF-MIP in cancer therapy through anti-angiogenesis and photothermal effect.



Scheme S2. The synthetic route of the VEGF epitope.



Scheme S3. The synthetic route of the hVEGF-MIP.

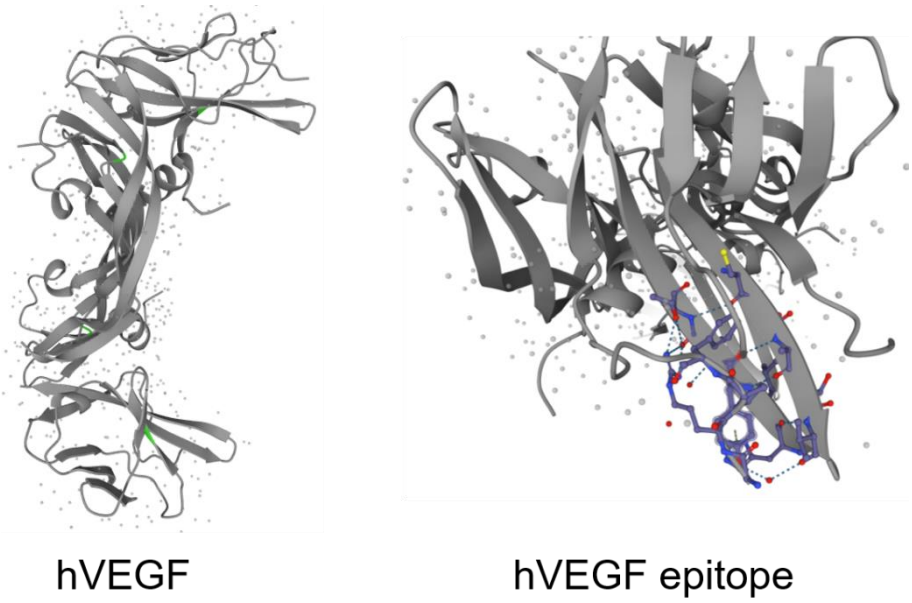


Fig. S1 Structure of human vascular endothelial growth factor (hVEGF) and the selected epitope. (From Uniport)

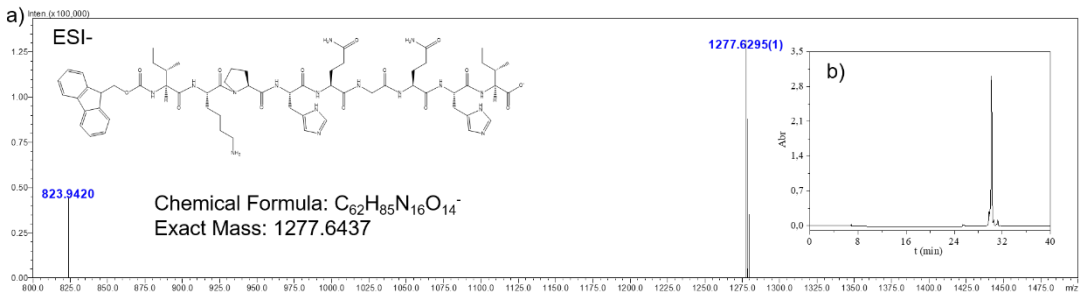


Fig. S2 (a) ESI mass spectrum of the VEGF epitope. (b) HPLC analysis of the VEGF epitope on a C18 column.

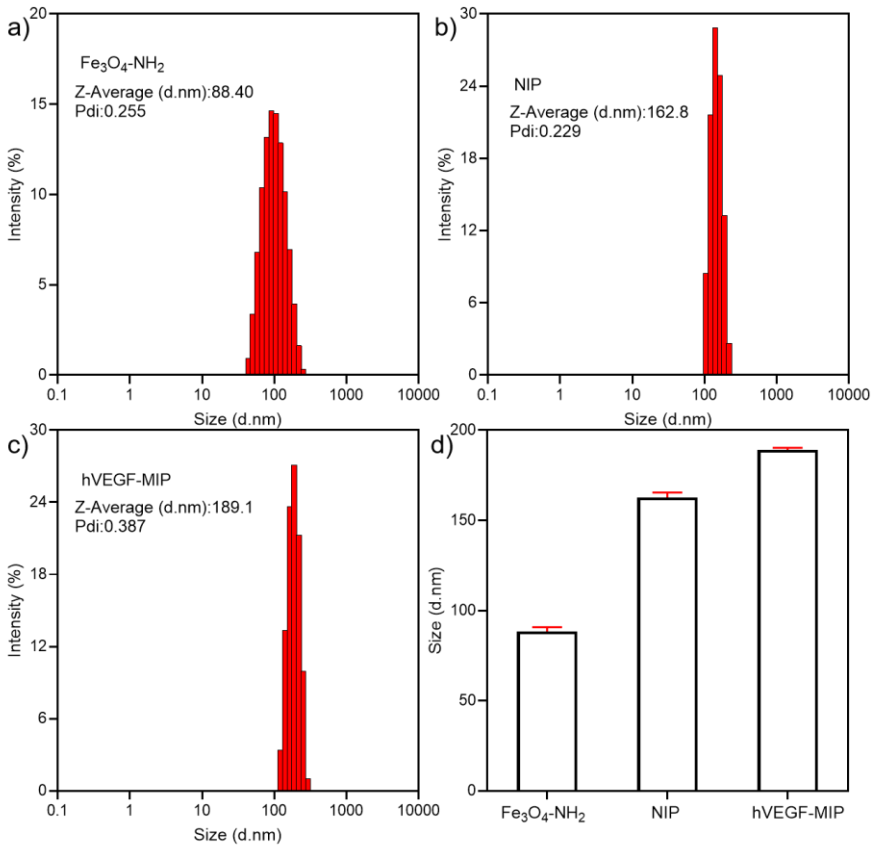


Fig. S3 DLS analysis of Fe_3O_4-NH_2 (a), NIP (b) and hVEGF-MIP (c). d) Particle size statistics analysis.

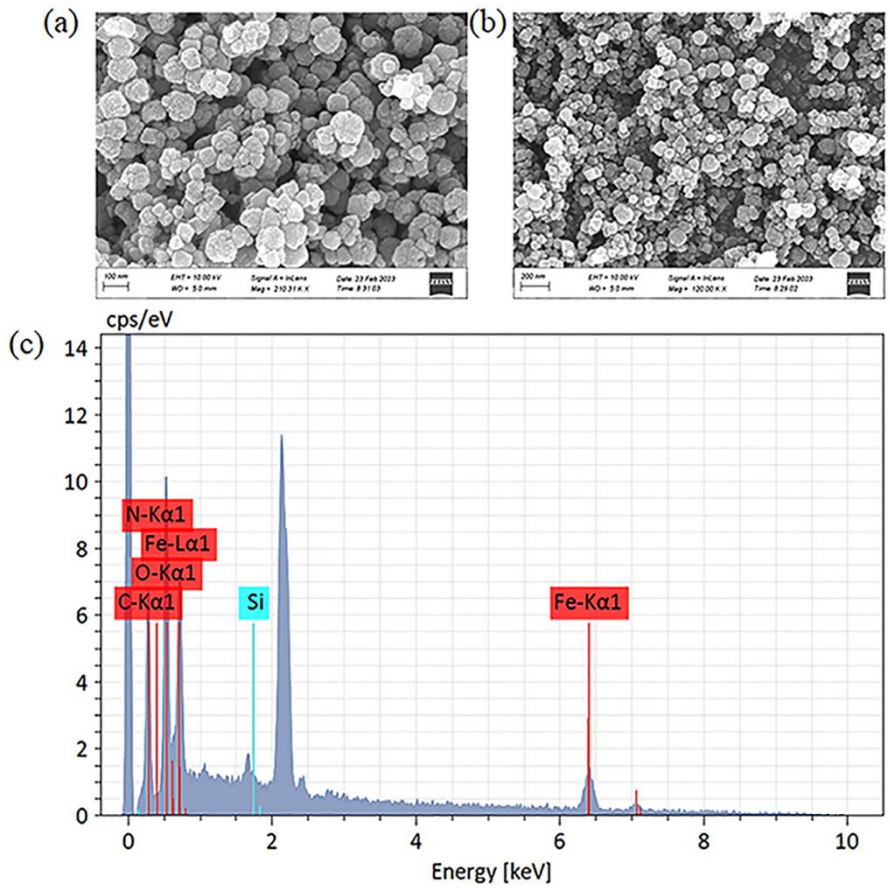


Fig. S4 (a)/(b) SEM image of hVEGF-MIP; (c) EDS data of hVEGF-MIP.

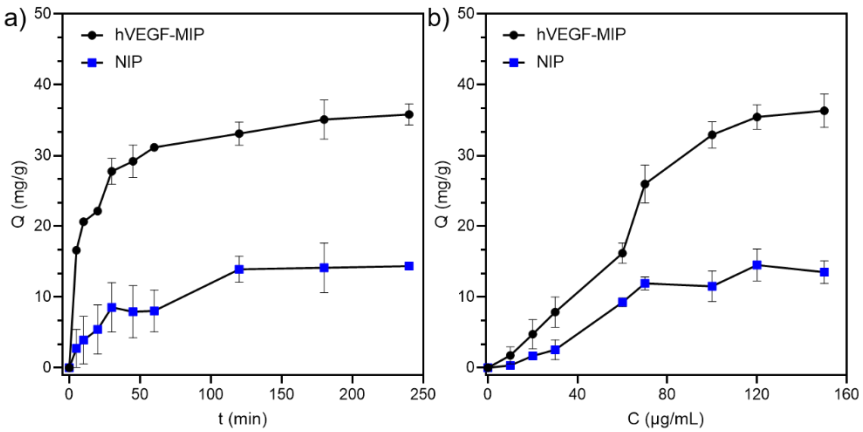


Fig. S5 a) Kinetic adsorption Curves of hVEGF-MIP and NIP; b) Isothermal adsorption Curves of hVEGF-MIP and NIP.

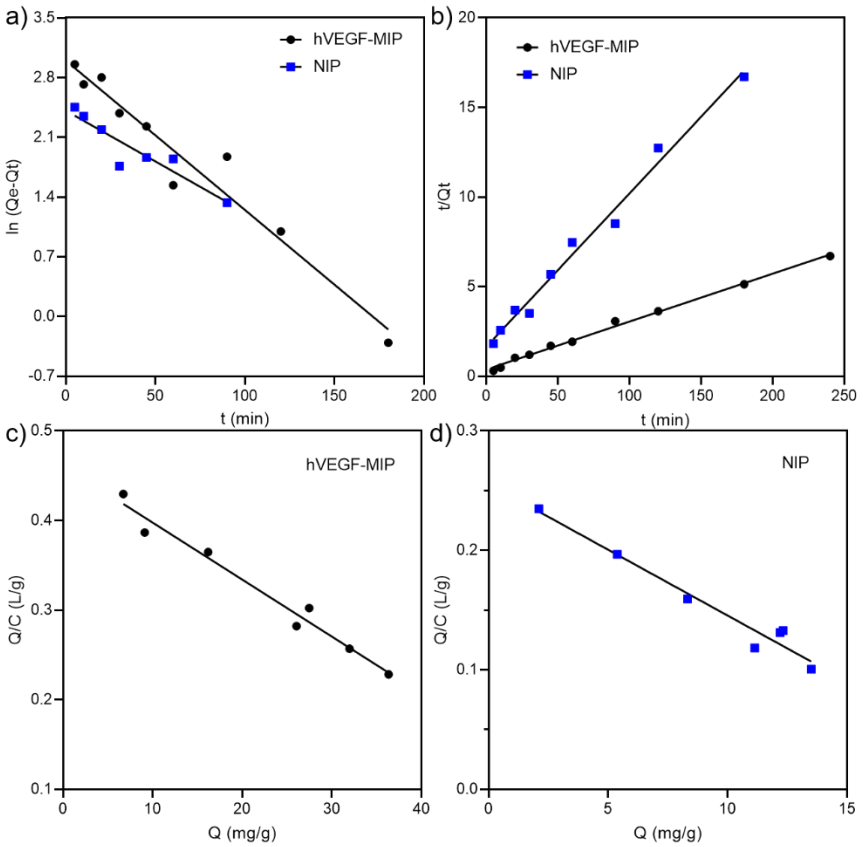


Fig. S6 hVEGF-MIP and NIP, a) and b) pseudo primary and pseudo secondary fitting curves; c) and d) Scatchard fitting curve.

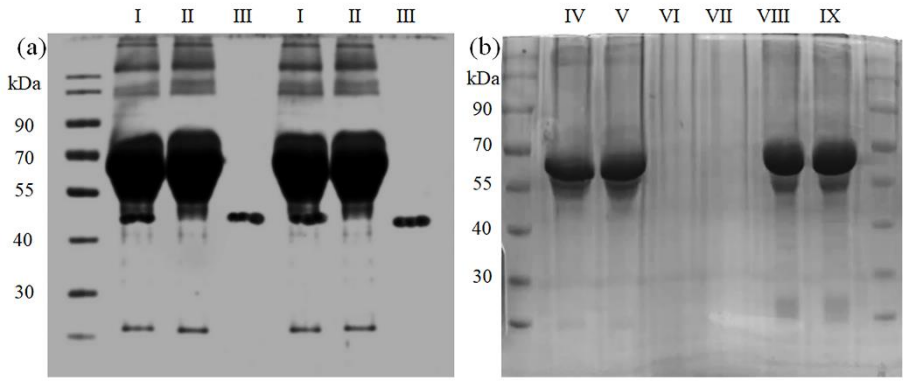


Fig. S7 SDS-PAGE protein analysis of cell lysate, supernatant, and hVEGF-MIP eluent, (a) Hela cell; (b) mouse 4T1 and B16 cell. (hVEGF MW:40-45 kDa; I, IV and IX: Hela, mouse 4T1 and B16 cell lysate, II, V and VIII: supernatant of hVEGF-MIP incubated with Hela, mouse 4T1 and B16 cell lysate for 4 h, III, VI and VII: eluent of hVEGF-MIP)

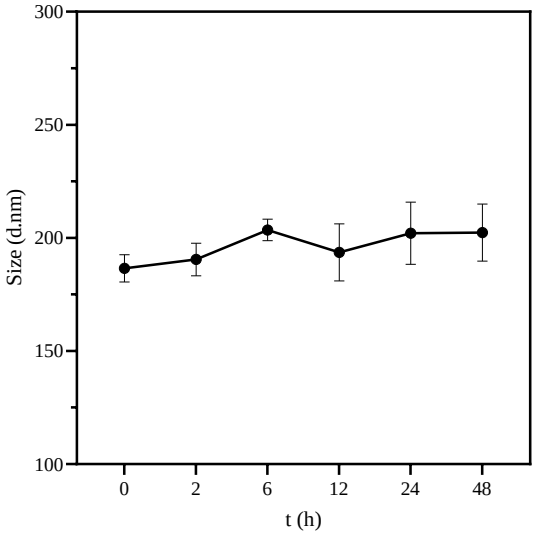


Fig. S8 The particle size changes of hVEGF-MIP in 10% serum environment.

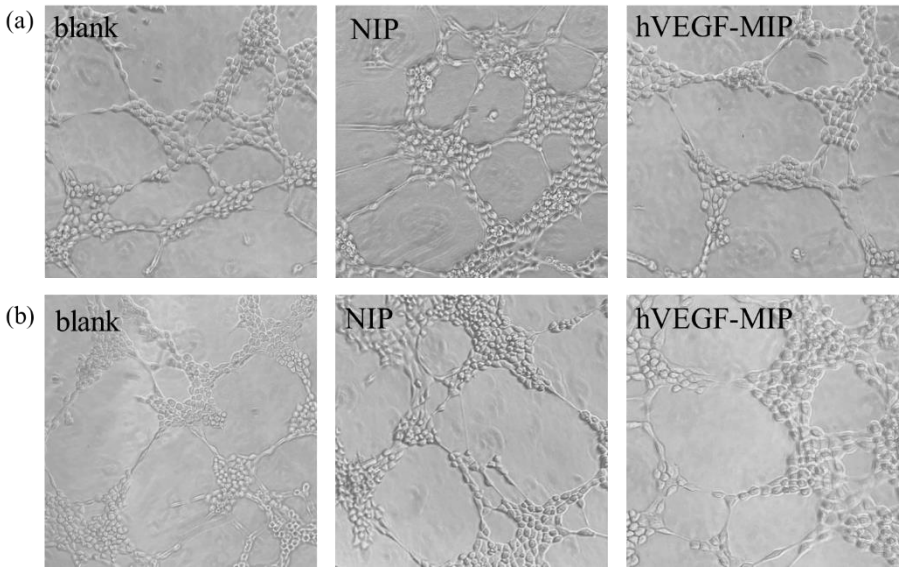


Fig. S9 The results of angiogenesis experiment in vitro (a) co-cultured the lysate supernatant of untreated and NIP/hVEGF-MIP-treated Hela cells with HUVEC cells; (b) co-cultured the supernatant of untreated and NIP/hVEGF-MIP-treated 4T1 cells with HUVEC cells.

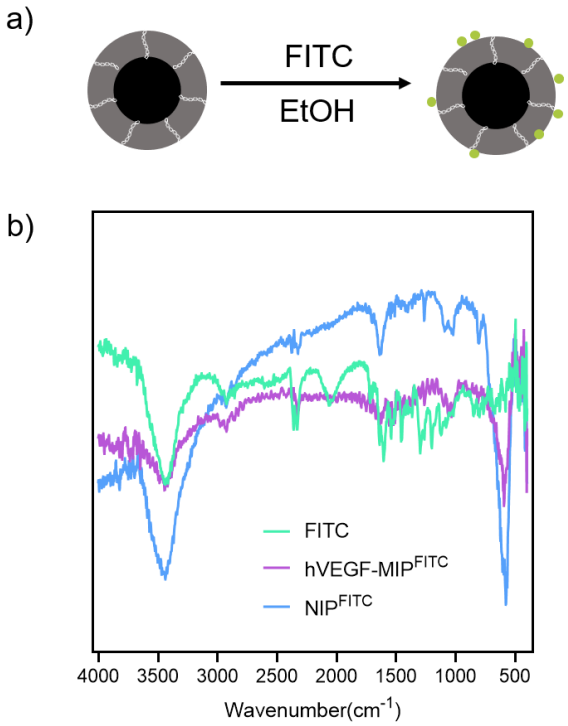


Fig. S10 a) The synthetic route of hVEGF-MIP^{FITC} and NIP^{FITC}; b) The FITR curves of FITC, hVEGF-MIP^{FITC} and NIP^{FITC}.

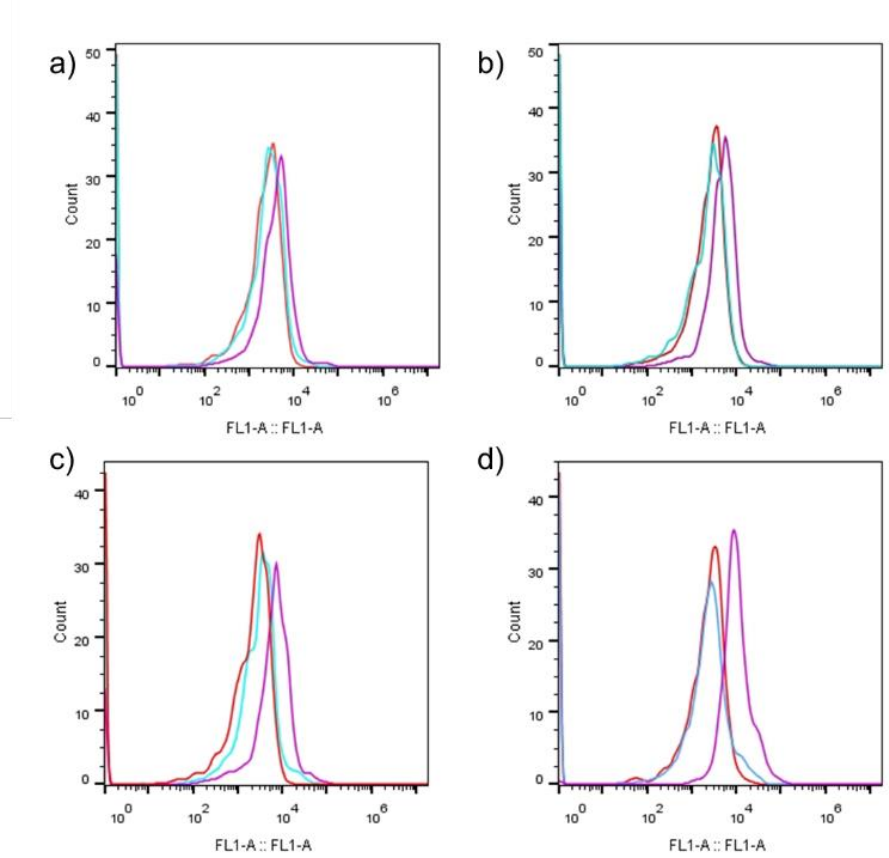


Fig. S11 cell uptake shifts of hVEGF-MIP and NIP at 200 μg/mL at different incubation times: a)3 h, b) 6 h, c) 12 h, d) 24 h (red line: Control; Blue line: hVEGF-MIP; Purple line: NIP)

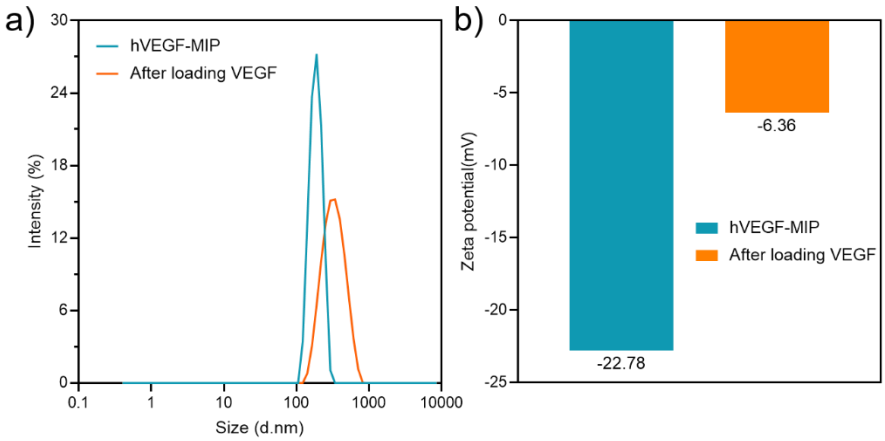


Fig. S12 Changes of a) DLS particle size and b) Zeta potential before and after hVEGF-MIP loading.

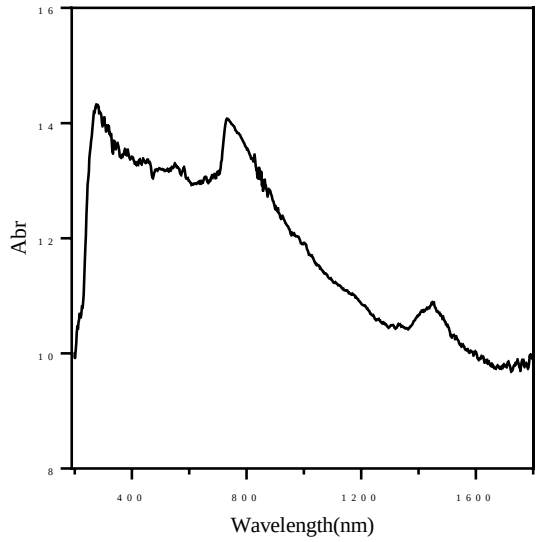


Fig. S13 The UV-Vis absorption spectrum of Fe_3O_4-NH_2.

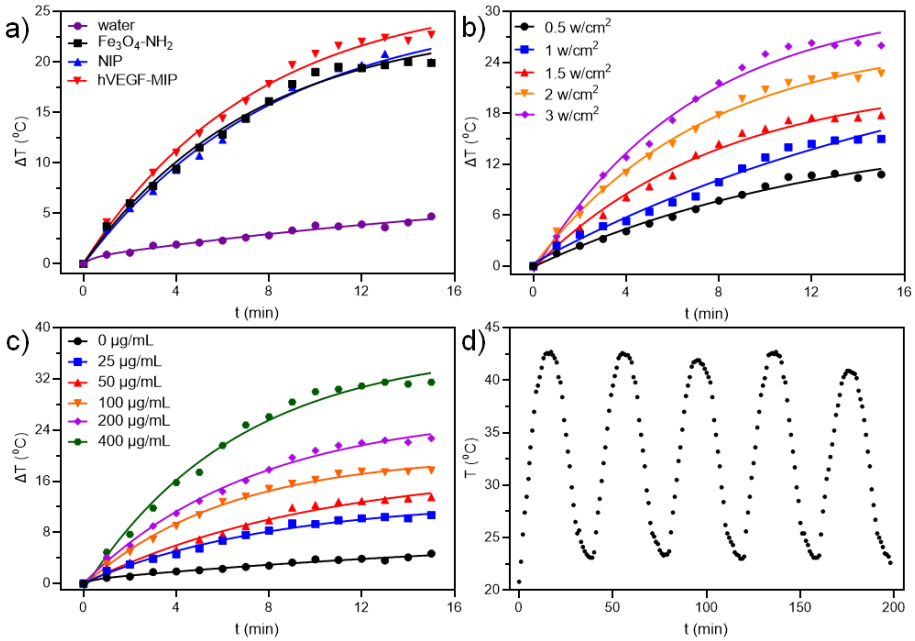


Fig. S14 Photothermal heating curves of a) Fe_3O_4-NH_2, hVEGF-MIP, NIP *aq* and pure water; hVEGF-MIP b) under various power densities, and c) with different concentrations; d) Heating/cooling curves of hVEGF-MIP for five cycles. (200 μg/mL, 808 nm, 2.0 W/cm^2)

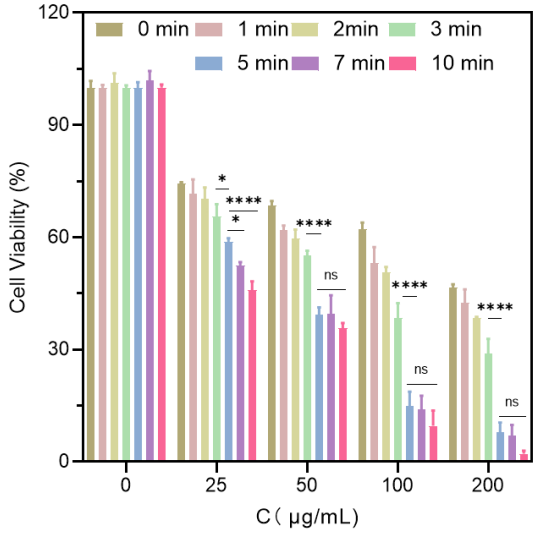


Fig. S15 Viabilities of HeLa cells irradiated with near-infrared laser for different time. (808 nm, 2 W/cm^2) (*/* */*** means p<0.005, **** stands for P<0.0001, ns means no significant difference)

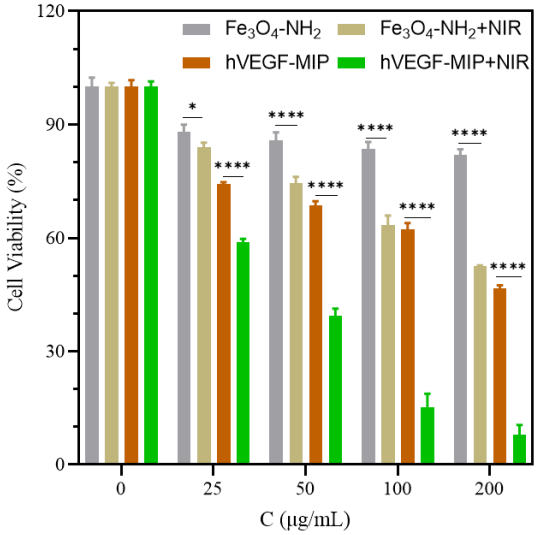


Fig. S16 Viabilities of HeLa cells under different concentrations of, Fe_3O_4-NH_2, hVEGF-MIP, Fe_3O_4-NH_2+NIR and hVEGF-MIP+NIR treatment (808 nm, 2 W/cm^2, 5 min) (*/* */*** means $p<0.005$, **** stands for $P<0.0001$)

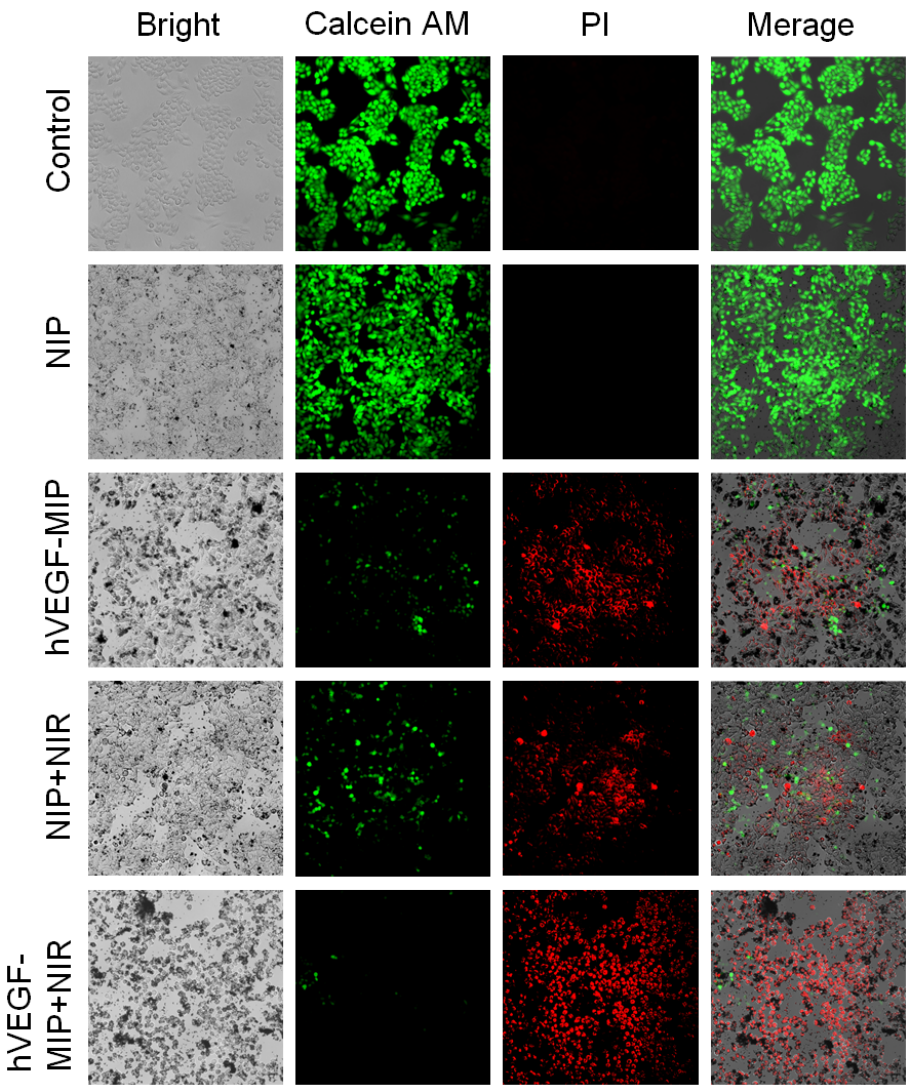


Fig. S17 HeLa cells were treated with Control, NIP, hVEGF-MIP, NIP+NIR and hVEGF-MIP+NIR, and then fluorescein imaging after co-staining with Calcein AM and Propidium iodide. (200 μg/mL, 808 nm,2 W/cm^2, 5 min)

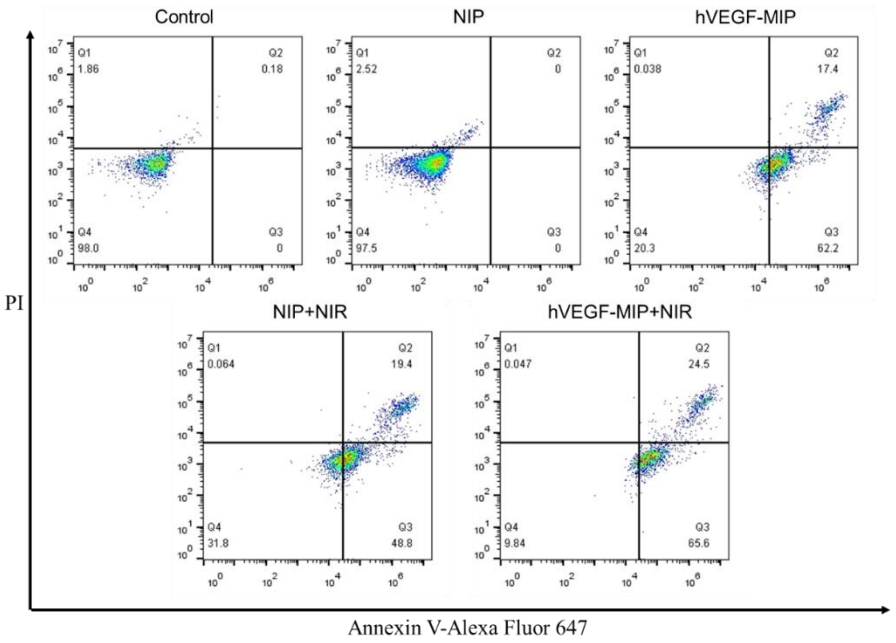


Fig. S18 Flow cytometry analysis of HeLa cells treated with hVEGF-MIP, NIP, hVEGF-MIP+NIR and NIP+NIR for 24 h. (200 μg/mL,808 nm, 2 W/cm^2, 5 min)

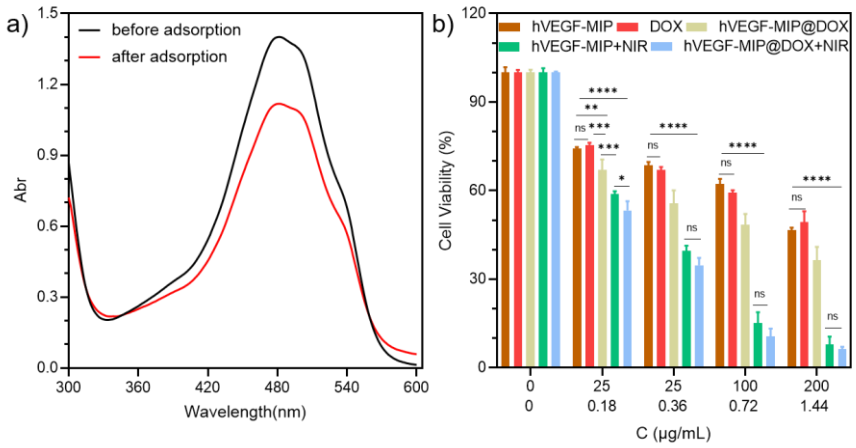


Fig. S19 a) Absorbance curve of DOX solution before and after treated with hVEGF-MIP; b) Viabilities of HeLa cells treated with different concentrations of DOX, hVEGF-MIP and hVEGF-MIP@DOX and irradiated with a near-infrared laser. (808 nm ,2 W/cm^2, 5 min), (*/* */*** means p<0.005, **** stands for P<0.0001, ns means no significant difference)

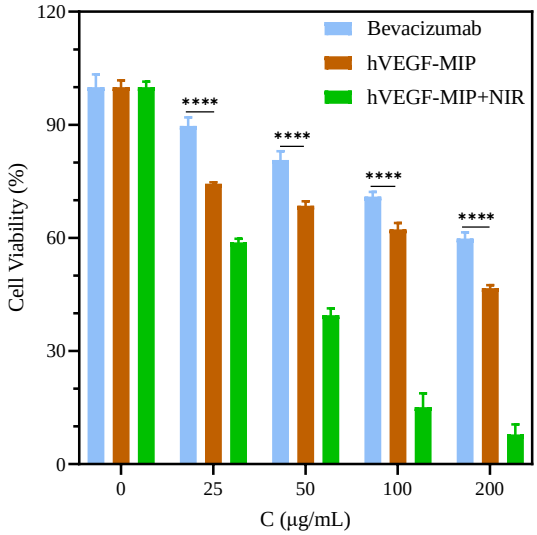


Fig. S20 Viabilities of HeLa cells under different concentrations of, Bevacizumab, hVEGF-MIP, and hVEGF-MIP+NIR treatment 24 h. (808 nm, 2 W/cm^2, 5 min) (**** stands for P<0.0001)

Table S1 The survival rate of different strategies applied in anti-angiogenesis (CCK-8)

Strategy	Drug name	Tumor cells	Survival rate %	Ref.
Direct use	Bevacizumab	A549 [a]	64.2%	5
	Endostar		50.3%	
	Apatinib	MCF-7 [b]	77.6%	6
	Lenvatinib		50.1%	
	Regorafenib		46.6%	
	Lenvatinib + Regorafenib	MDA-MB-231 [c]	25.4%	
			70.8%	
Nanocarrier	RRRKRR	MDA-MB-231	34.3%	7
	Bis-pyrene-KLVFF-RRRKRR	U87 [d]	20.5%	8
		U251 [d]	40.3%	
	Icaritin + Coix seed oil	HepG-2 [e]	40.7%	9
	Itraconazole+siRNA^{VEGF}	4T1 [f]	52.6%	10
	hVEGF-MIP	HeLa [g]	14.7%	Here
		HepG-2	23.4%	
		HCT-116 [h]	22.5%	

[a] A549 cell, human alveolar adenocarcinoma cell;

[b] MCF-7 cell, human breast cancer cell;

[c/f] MDA-MB-231/4T1 cell, mouse breast cancer cell;

[d] U87 and U251 cell, glioma cell;

[e] HepG-2 cell, human hepatoma cell;

[g] HeLa cell, a human cervical cancer cell;

[h] HCT-116 cell, human colorectal cancer cell.

Table S2 Kinetics absorption experiment of hVEGF-MIP and NIP

	Kinetic model	Linear regression	K(g/(mg/min))	Qe(mg/g)	R^2
hVEGF-MIP	Pseudo-first-order	$y=-1.75\times10^{-2}x+3.00$	1.75×10^{-2}	20.1	0.941
	Pseudo-second-order	$y=2.67\times10^{-2}x+0.379$	1.88×10^{-3}	37.4	0.994
NIP	Pseudo-first-order	$y=-1.19\times10^{-2}x+2.41$	1.19×10^{-2}	11.1	0.839
	Pseudo-second-order	$y=8.56\times10^{-2}x+1.64$	4.45×10^{-3}	11.7	0.985

Table S3 The parameters of the Scatchard equation

	Scatchard curve	**R^2**	**K_d (mg/L)**	**Q_{max} (mg/g)**
hVEGF-MIP	$y=-6.64\times10^{-3}x+0.461$	0.968	157	72.5
NIP	$y=-1.10\times10^{-2}x+0.256$	0.951	90.8	23.2

Table S4 The inhibition rate of different strategies applied in photothermal synergistic

Regent	Conditions	Cell	Inhibition rate %				Ref.
			PTT	chemotherapy	anti-angiogenesis	Synergetic therapy	
IR825@B-PPNs	2 mg/mL, 825 nm, 0.8 W/cm^2, 5 min	C643[a)]	85.2%	-	40.3%	89.3%	11
RAPA/PFBT-HGCNs	74 μg/mL, 670 nm, 1 W/cm^2, 3 min	4T1	74.5%	49.4%	-	80.98%	12
CPAP NPs	200 μM, 808 nm 1 W/cm^2, 15 min	CT26[b)]	72.2%	61.2%	-	89.6%	13
DOX@MSN-WS_2-HP	4 μg/mL, 808 nm 1 W/cm^2, 10 min	4T1	61.4%	63.3%	-	86.5%	14
CA4-ND@PS	10 μg/mL, 808 nm 2 W/cm^2, 5 min	HepG-2	11.4%	-	47.6%	89.2%	15
Bevacizumab	200 μg/mL	Hela	-	-	40.2%	-	Here
hVEGF-MIP	200 μg/mL, 808 nm 2 W/cm^2, 5 min	Hela	53.2%	-	85.3%	92.1%	Here
		HepG-2	-	-	76.6%		
		HCT-116	-	-	75.5%		

[a)] C643 cell, anaplastic thyroid carcinoma cell;

[b)] CT26 cell, mouse colorectal cancer cell.

References

1. (a) A. E. Nezir, M. P. Khalily, S. Gulyuz, S. Ozcubukcu, S. G. Kucukguzel, O. Yilmaz, D. Telci, *Amino Acids*., 2021, **53**, 645-652. (b) I. N. Sabana, Muhammad. Wiani, Ika. Zainuddin, Achmad. Hidayat, Ace. Harneti, Desi. Nurlelasari, Nurlelasari. Al-Anshori, Jamaludin. Supratman, Unang. Maharani, Rani, *Egypt. J. Chem.*, 2019, **63**, 921-926. (c) H. Hou, Y. Jin, K. Xu, L. Sheng, Y. Huang, R. Zhao, *Anal. Chim. Acta*, 2021, **1154**, 338301.
2. L. Wang, J. Bao, L. Wang, F. Zhang, Y. Li, *Chem. Eur. J.*, 2006, **12**, 6341-6347.
3. W. S. Zou, Y. Q. Wang, F. Wang, Q. Shao, J. Zhang, J. Liu, *Anal. Bioanal. Chem*., 2013, **405**, 4905-4912.
4. (a) A. Arora, A. M. Kivela, L. Wang, R. Minkeviciene, J. H. Taskinen, B. Zhang, A. Koponen, J. Sun, M. Shirane, Y. Zhou, P. Hotulainen, C. Raiborg, V. M. Olkkonen, *Cell. Mol. Life Sci.*, 2022, **79**, 220. (b) G. Zhang, T. Wang, Z. Huang, Y. Chen, L. Sun, X. Xia, F. He, C. Fan, S. Wang, W. Liu, *Horm. Cancer*, 2022, **13**, 89.
5. Y. Jin, L. Wei, Q. Jiang, X. Song, C. Teng, C. Fan, Y. Lv, Y. Liu, W. Shen, L. Li, D. Huang, T. Xin, *Sci Rep.*, 2018, **8**, 15837.
6. K. Bajbouj, R. Qaisar, M. A. Alshura, Z. Ibrahim, M. B. Alebaji, A. W. Al Ani, H. M. Janajrah, M. M. Bilalaga, A. I. Omara, R. S. Abou Assaleh, M. M. Saber-Ayad, A. B. Elmoselhi, *Int. J. Mol. Sci.*, 2022, **23**, 4408.
7. B. N. Li, P. P. He, P. P. Yang, J. P. Zhang, L. Wang, H. Wang, *J. Mater. Chem. B.*, 2018, **6**, 5282-5289.
8. J. Wang, Y. Yang, Y. Zhang, M. Huang, Z. Zhou, W. Luo, J. Tang, J. Wang, Q. Xiao, H. Chen, Y. Cai, X. Sun, Y. Wang, Y. Ke, *Adv. Funct. Mater.*, 2016, **26**, 7873-7885.
9. J. Guo, H. Zeng, Y. Liu, X. Shi, Y. Liu, C. Liu, Y. Chen, *Int. J. Pharm.*, 2021, **601**, 120533.
10. M. Jin, B. Zeng, Y. Liu, L. Jin, Y. Hou, C. Liu, W. Liu, H. Wu, L. Chen, Z. Gao, W. Huang, *Pharmaceutics*, 2022, **14**, 1369.
11. Q. Wang, G. Sui, X. Wu, D. Teng, L. Zhu, S. Guan, H. Ran, Z. Wang, H. Wang, *Acta Biomater.*, 2020, **102**, 367-383.
12. X. Pang, X. Tan, J. Wang, L. Liu, Q. You, Q. Sun, Y. Wang, F. Tan, N. Li, *Adv. Healthcare Mater.*, 2017, **6**, 1700099.

13. W. Zhong, K. H. Wong, F. Xu, N. Zhao, M. Chen, *Acta Biomater.*, 2022, **145**, 135-145.
14. Q. Lei, S. B. Wang, J. J. Hu, Y. X. Lin, C. H. Zhu, L. Rong, X. Z. Zhang, *ACS Nano*, 2017, **11**, 7201-7214.
15. Y. Li, J. Lu, X. Deng, X. Wang, F. Jia, S. Zhong, X. Cui, Z. Pan, L. Shao, Y. Wu, *Nanotechnology*, 2021, **32**, 5101.