Supporting Information

Metal Ions Driven Assembly for Constructing Metal–Phenolic Network Nanoparticles Loaded Hydrogel as Tumor Photothermal-Immunotherapy Agent

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Experimental section

Materials

Gel, PA and ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, ≥98%) were supplied by Sinopharm Chemical Reagent Co., Ltd. RPMI 1640 medium, Dulbecco's Modified Eagle's medium (DMEM), fatal bovine serum (FBS), Penicillin-Streptomycin Solution (PS), trypsin, Calcein-AM (CA) and Propidium Iodide (PI) stain agent, Cell Counting Kit-8 (CCK-8 assay) agent, Anti-calreticulin (CRT),Anti-high mobility group box-1 (HMGB1) protein, ATP Content Assay Kit and Annexin V-FITC Apoptosis Detection Kit were purchased from Solarbio Science & Technology Co.,Ltd. (Beijing, China). Mouse breast cancer (4T1) and mouse fibroblast (L929) were obtained from American Type Culture Collection.

Preparation of GMP hydrogel

Gel, PA and $FeCl_3 \cdot 6H_2O$ were dissolved in DI water at the designed concentration of 100 mg/mL, 1 mg/mL and 10 mg/mL respectively. GMP hydrogel was obtained via adding 100 μL PA solution and 200 μL $FeCl_3 \cdot 6H_2O$ solution into 0.5 mL Gel. Afterward, the composite was mixed using vortex device for about 10 seconds and then placed at room temperature. The process was consistent with above procedures and then final Gel-PA and Gel-Fe were obtained. The composite could be freeze dried to save or further characterize and used.

Rheological testing of obtained hydrogel

In order to investigate the influence of Fe^{3+} metal ions on Gel hydrogel formation, a series of Gel-Fe samples were prepared via adding different volume $FeCl_3 \cdot 6H_2O$ solution (25 μL, 50 μL, 100 μL, 200 μL) into Gel solution (0.5 mL, 10% concentration), which were named as Gel-Fe25, Gel-Fe50, Gel-Fe100, Gel-Fe200. The samples were tested at 37℃ and a strain of 1% condition for rheological

properties.

Photothermal property GMP hydrogels

The photo-induced heat effect of Gel, Gel-PA, Gel-Fe and GMP hydrogel were tested according to the following procedures. 100 μL PA solution (1 mg/mL) and 200 μL $FeCl_3 \cdot 6H_2O$ solution (10 mg/mL) were added into Gel solution (0.5 mL, 10% concentration) to prepare GMP hydrogel, which was named as $GM_{10.0}P_{1.0}$. Similarly, the samples of $GM_{20.0}P_{1.0}$, $GM_{5.00}P_{1.0}$, $GM_{1.25}P_{1.0}$, $GM_{10}P_{4.0}$, $GM_{10}P_{2.0}$, $GM_{10}P_{1.0}$, $GM_{10}P_{0.5}$, $GM_{10}P_{0.25}$, $GM_{10}P_{0.125}$, $GM_{10.0}P_4$, $GM_{5.00}P_4$, $GM_{2.50}P_4$, $GM_{1.25}P_4$, $GM_{0.62}P_4$, $GM_{0.31}P_4$ could also be obtained. Hydrogel (1 mL) was placed in tube and then irradiated by 808 nm laser for different minutes under different power density (0.5, 1.0, 1.5 and 2.0 W/cm^2). The temperature change was recorded by IR cameras (with an accuracy of 0.1℃) at 30 seconds interval and analyzed by original software.

Cell culture and animal model

L929 and 4T1 were purchased from American Type Culture Collection (ATCC) and cultured in RPMI 1640 (RPMI 1640 base medium 89%, FBS 10%, PS 1%) and DMEM completed medium (DMEM base medium 89%, FBS 10%, PS 1%), respectively. Balb/c mice with 4T1 tumor-bearing breast were selected to fabricate residual tumor model and bilateral tumor model. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Tsinghua University and approved by the Animal Ethics Committee of Tsinghua University (Project No. 24-ZLY1, Tsinghua University, Beijing, China).

In vitro cell biocompatibility

Firstly, L929 cells and 4T1 cells were seeded in 96-well plate with 200 μL/well at a density of 2.5×10^4 cells/mL for incubation 24 h. Then, completed condition medium with different GMP

hydrogel concentrations (10, 5, 2.5, 1.25, 0.625 mg/mL) was added into L929 and 4T1 well after the remove of cell medium. After another 24 h incubation, the medium was removed to add 100 μL CCK 8 agent (condition base medium : CCK-8 = 9:1) into well to incubate for 2 h. Finally, 80 μL CCK-8 agent was collected to detect at 450 nm using microplate reader device.

In vitro cell cytotoxicity assay

Firstly, 4T1 cells were seeded in 96-well plate and incubated in cell incubator for 24 h at 37 °C in the 5% CO_2. Afterwards, the hydrogel was injected on the bottom of 96-well plate with circle shape, which was then irradiated by 808 nm laser at different power density (0.5, 1.0, 1.5 and 2.0 W/cm^2). Finally, the CCK-8 assay was used to evaluate hydrogel-induced photothermal cellular killing effect. Furthermore, the treated cells were also stained by Calcein-AM and Propidium Iodide for live/dead observation.

Immunofluorescence analysis of CRT and HMGB1

4T1 cells was cultured in confocal dish for 24 h. Then, the cells were cultured with Gel-PA, Gel-Fe and GMP. In GMP group, the cells were irradiated by 808 nm laser for two minutes. Afterwards, Gel-PA, Gel-Fe and GMP were removed and cells were cultured for another 12 h. Subsequently, after being washed with PBS, the cells were fixed with 4% paraformaldehyde for 20 minutes and then permeabilized with 0.1% Triton-100 for 10 minutes. Subsequently, cells were blocked with 5% BSA for 30 minutes and cultured with anti-CRT or anti-HMGB1 for overnight at 4℃. Then the cells were stained with FITC-labeled second antibody for 1 h. Finally, the cells were stained with Hoechst 33342 and observed by CLSM.

Flow cytometry analysis

The cell apoptosis induced by GMP+NIR treatment was investigated using an Annexin V-FITC/PI

apoptosis detection kit. 4T1 cells were collected at GMP hydrogel-loaded tube and irradiated by 808 nm laser for 3 min at 2.0 W/cm^2. The cells were then spread on cell dish for 6 h. The supernatant was collected to incubate extracted dendritic cells (DCs), which was then labeled with CD11c，CD80，CD86 for Flow cytometry analysis. Moreover, the 4T1 cells were collected again and stained with Annexin V-FITC/PI for Flow cytometry analysis.

In vivo bio-safety

The histocompatibility of hydrogel was investigated using Balb/c mice. The mice were subcutaneously implanted with hydrogel. At a designed time point of 3, 7, 14 and 21 days, the main organs including skin, heart, liver, spleen, lung, and kidney were collected for H&E staining.

In vivo local antitumor efficiency

The suspension of 4T1 cells were incubated under the right side armpit skin of Balb/c mice. When the tumor volume reached about 100 mm^3, ≈3/4 tumor tissue was removed via surgery method and then the left ≈1/4 tumor tissue was used to simulate the residual tumor bed. Afterwards, the mice were randomly divided into five groups including (i) surgery group, (ii) surgery + Gel, (iii) surgery + Gel-Fe (iv) surgery + GMP and (v) surgery + GMP + NIR (1.5 W/cm^2, 5 min). The body weight and tumor volume of mice were collected and recorded every two days after different treatments. After 13 day, the mice were sacrificed to collect the main organs (heart, liver, spleen, lung and kidney) and tumor tissue. The tissue sections were stained with H&E for histological analysis.

In vivo immune response effect evaluation

The bilateral 4T1 tumor model was established to evaluate in vivo immune response. Firstly, the 4T1 cells were incubated under right side armpit skin of Balb/c mice to fabricate primary tumor tissue. After 5 days, the 4T1 cells were injected into left side armpit skin to fabricate distant tumor tissue.

Then after another 3 days, the mice were divided into three groups including control group, GMP group and GMP + NIR (1.5 W/cm^2, 5 min) group. Then the GMP hydrogel was subcutaneously implanted into primary tumor tissue and then irradiated by 808 nm laser. The body weight and tumor volume (left and right) were collected and recorded. After 13 day, the mice were sacrificed to collect blood serum for tumor necrosis factor (TNF-α) and interferon-γ (IFN-γ) analysis. Meanwhile, the main organs (heart, liver, spleen, lung and kidney) were collected to prepare sections for H&E staining. Additionally, the tumor tissue (primary and distant) were obtained to prepare tumor sections for histological analysis (H&E, CD4 and CD8).

Statistical analysis

All data were presented as the mean ± standard error (S.E.). The comparison between different treatments was conducted using Student's t-test. Statistical significance was indicated for P values reaching *P < 0.05, **P < 0.01, and ***P < 0.001

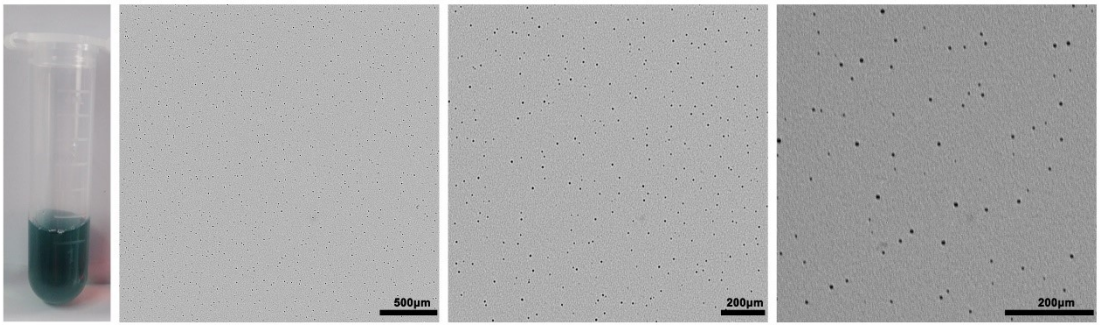

Fig. S1. Photography of reaction produce between tannic acid and Fe^{3+} metal ions with TEM images observation.

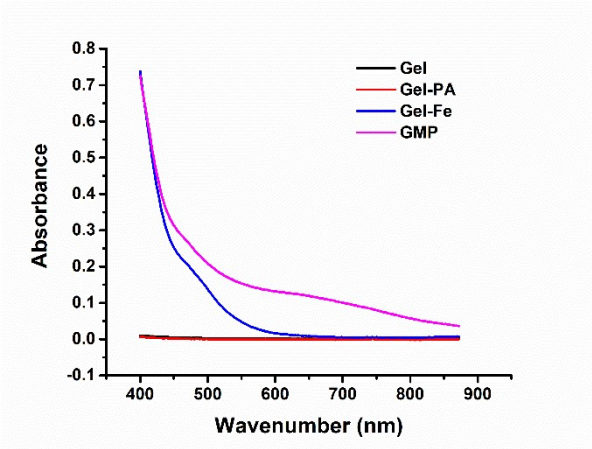

Fig. S2. Absorption curve of Gel, Gel-PA, Gel-Fe and GMP hydrogel

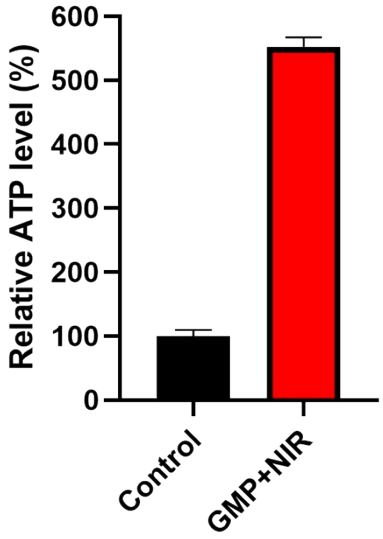

Fig. S3. ATP test from supernatant of 4T1 cells with or without GMP + NIR treatment

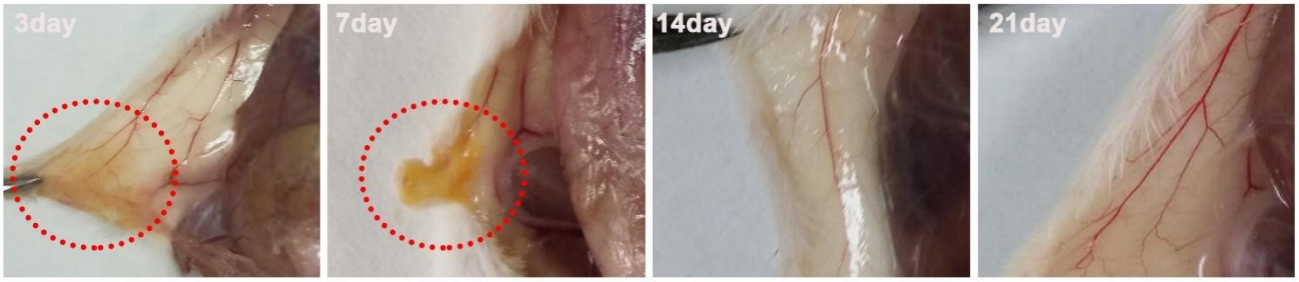

Fig. S4. Images of subcutaneous GMP hydrogel degradation behavior at 3 days, 7 days, 14 days and 21 days.

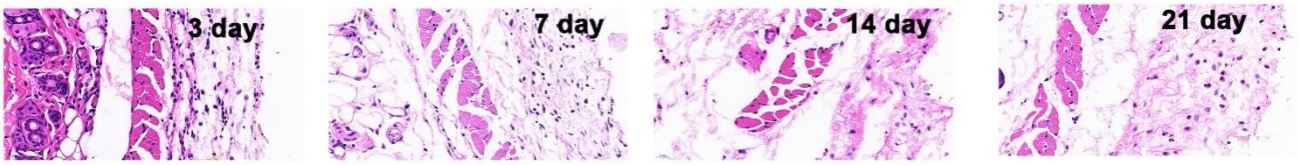

Fig. S5. H&E staining of skin tissue from GMP hydrogel injection area at 3 days, 7 days, 14 days and 21 days.

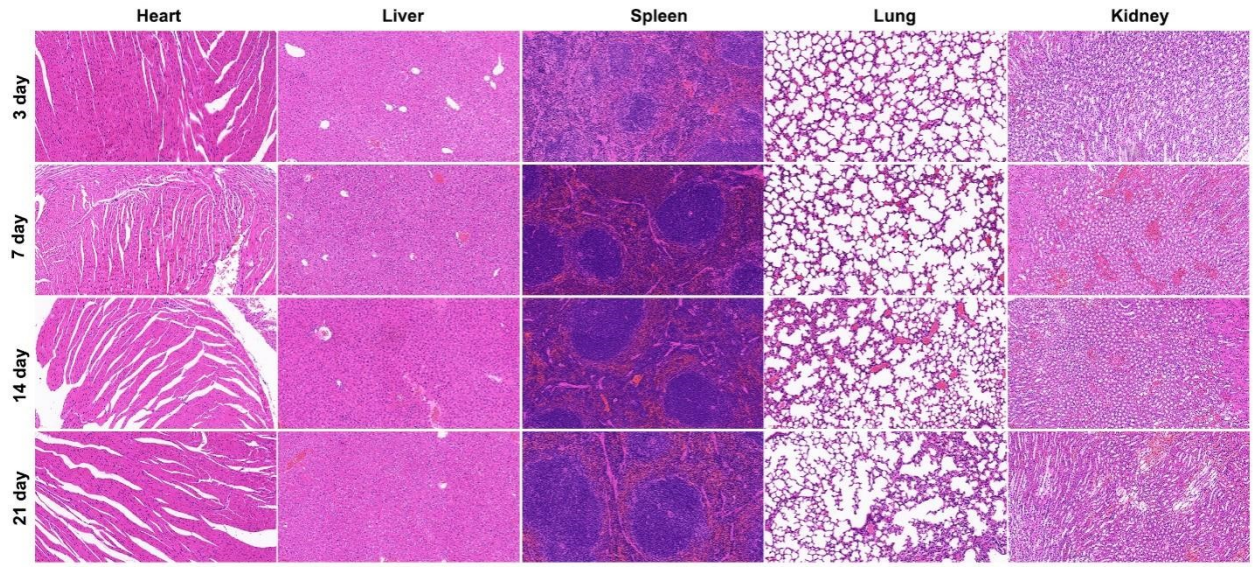

Fig. S6. H&E staining of main organs (heart, liver, spleen, lung and kidney) after GMP hydrogel injection at 3 days, 7 days, 14 days and 21 days.