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Supplementary Information

The Biological Response of Based pH-switch Gold Nanoparticle-Composite Polyamino Acid Embolic Material

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1. Design concept of materials

Peptide polymers possess numerous remarkable features, including well-established synthesis methods, high biocompatibility, superior degradability, and targeted capabilities. Research indicates that different amino acids within peptide polymers exhibit distinct pH responsiveness due to the ionization properties of their side chains. The varying charge states of different amino acid side chains under different pH conditions can impact the solubility, morphology, and properties of the entire polymer,

enabling a sol-gel transition in response to pH changes. Exploiting this feature, this study developed a two-segment polymer: poly(L-Glutamic acid-L-Tyrosine) (PLGTs), to achieve pH-responsive functionality. The introduction of methoxy polyethylene glycol amine (*m*PEG-NH_2) enhances the water solubility of PLGTs, facilitating the formation of a water gel. The polymer *m*PEG-PLGTs exhibits different pH responses by adjusting the ratio of L-Glutamic acid to L-Tyrosine. Additionally, this study utilized L-Cysteine-modified Au NPs, conferring fluorescent properties. Through electrostatic adsorption, Au NPs and *m*PEG-PLGTs were coalesced, imparting the material with fluorescent labeling capabilities.

2. Data availability

The data used to support the findings of this study are available from the corresponding author on request.

3. Hemolysis test

According to the national standards GB/T 16886.12-2005 and GB/T 14233.2-2005, the procedure begins by obtaining 1mL of whole blood from adult female SD rats and diluting it with 2mL of PBS. Following centrifugation and three washes, the red blood cells are separated and diluted to 5mL with PBS solution. For the positive control group, 0.2mL of whole blood is mixed with 0.8mL of distilled water; for the negative control group, 0.2mL of whole blood is mixed with 0.8mL of PBS buffer solution. Four gradient concentrations of sample groups are then prepared. After incubating each sample at 37°C for 1 hour, the supernatant is collected and added to a 96-well plate for

labeling. Finally, the absorbance of each well is measured using a microplate reader at a wavelength of 570nm, and the hemolysis rate is calculated using the following formula: $hemolysis\ rate(\%) = (A_p - A_b)/(A_t - A_b) \times 100\%$, here, A_p: Sample group; A_t: Positive control group; A_b: Negative control group.

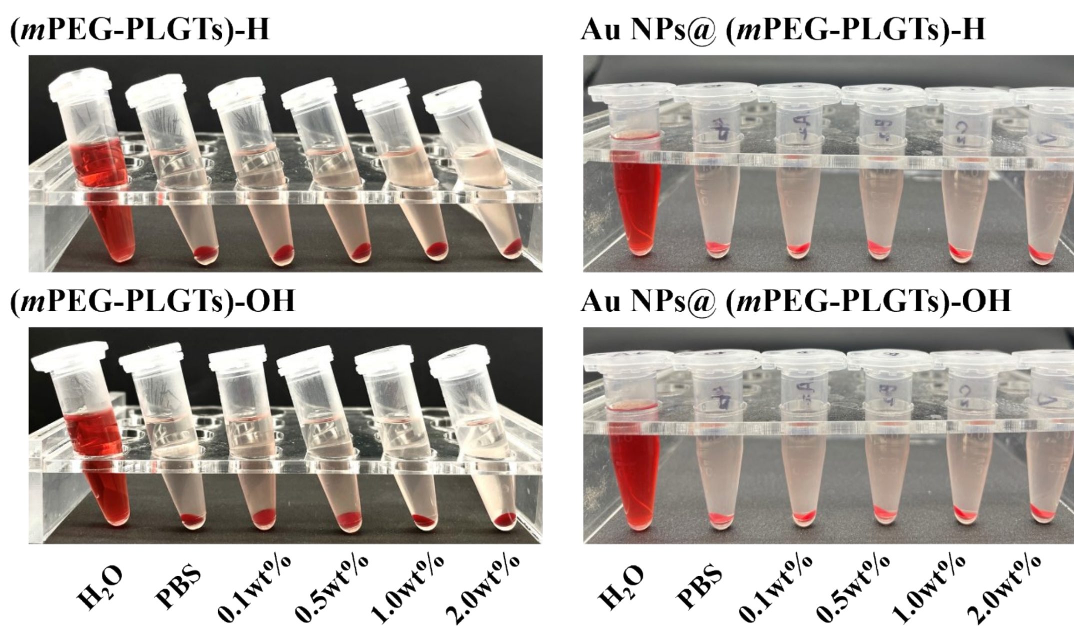

Fig. S1: The photographs after the red blood cell incubation with *m*PEG-PLGTs and Au NPs@(*m*PEG-PLGCs).

4. Coagulation study.

Collect 10 mL whole blood from New Zealand white rabbits and centrifuge to obtain platelet-poor plasma (PPP). Mix 450 μL PPP with 50 μL of the sample or PBS, and incubate at 37°C for 30 minutes. Subsequently, use an automated coagulation analyzer to measure the activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT), and fibrinogen (FIB) levels in the incubated PPP. Specifically, perform the APTT test by incubating plasma with Actin reagent followed by the

addition of $CaCl_2$ solution to measure clot formation time. For the PT test, mix plasma with Thromborel S reagent to measure coagulation time. Conduct the TT test by mixing plasma with thrombin time determination reagent to determine clotting time. Finally, perform the FIB test by mixing plasma with fibrinogen test reagent to measure fibrinogen levels. Record and analyze the numerical values of each coagulation parameter and compare the differences between the sample group and the control group.

5. Cytotoxicity evaluation.

This study utilized the CCK-8 assay to assess the cytotoxicity of the polymer on mouse fibroblast cells (L929). In a 96-well plate, each well was seeded with 1×10^5 L929 cells in 100 μL of 10% fetal bovine serum (FBS) Dulbecco's Modified Eagle Medium (DMEM). After 24 hours of incubation in a CO_2 cell culture incubator, the tested samples were dissolved in 10% FBS DMEM and added to the plate, with 5 wells allocated for each sample as parallel replicates. Samples were tested at four final concentration gradients (10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.5 mg/mL), along with a control group containing only 10% FBS DMEM and a blank control group with no cells. Subsequently, the cells were further incubated for 24, 48, and 72 hours. At each time point, the culture medium was replaced with 10% CCK-8 DMEM solution and incubated for an additional 2 hours in the dark. Finally, absorbance at 450 nm was measured using a microplate reader, and absorbance values at 24, 48, and 72 hours of incubation were recorded. Cell viability was calculated using the formula provided: $Cell\ viability(\%) = (OD_t - OD_c)/(OD_0 - OD_c) \times 100\%$, here, OD_t:denotes the absorbance of the experimental group; OD_c: represents the absorbance of the blank

control group; and OD_0:signifies the absorbance of the DMEM control group.

6. Cell viability assay

According to the described procedure, culture L929 cells for 24 hours, 48 hours, and 72 hours. At each time point, remove the culture medium and add the working solution of Calcein AM and PI. Incubate the cells in the CO_2 incubator for 30 minutes in the absence of light. After incubation, observe the staining effect using a fluorescence microscope: Calcein AM will display green fluorescence, allowing for the recording of live cell counts, while PI will display red fluorescence, facilitating the recording of dead cell counts.

7. Degradability test.

This experiment utilized a dialysis method to evaluate the degradation kinetics and extent of *m*PEG-PLGTs when exposed to a composite proteinase aqueous solution. A suitable amount of *m*PEG-PLGTs and composite proteinase was placed within a dialysis membrane with a molecular weight cutoff of 3000, while a control group without composite proteinase served as a blank (n=5). The samples were agitated at 37°C, and at 48-hour intervals, the remaining solid *m*PEG-PLGTs within the dialysis bag were retrieved. Following three washes with 4 mL of ultrapure water, the samples underwent a 24-hour drying period at 37°C in a vacuum oven before being weighed and recorded. This process continued until either the solid mass ceased to decrease or the polymer disappeared from the dialysis membrane. The percentage mass loss was calculated using the formula: $Mass\ loss(\%) = (w_0 - w_t)/w_0 \times 100\%$, here, w_0:

represents the initial mass; w_t: signifies the remaining mass at different time points.

8. Establishment of mouse tumor model

In sterile conditions, culture the 4T1 cell line, wash them with PBS, and lyse them to obtain a single-cell suspension. Count the cells and adjust the cell density to an appropriate concentration. Inject the 4T1 cell suspension (3×10^5 cells/100μL) into the left side of healthy female Balb-c mice. Monitor the mice's weight and tumor growth regularly after injection.

9. Fluorescent tracing

Fluorescence monitoring was conducted using near-infrared (NIR) excitation (EX: 600nm; EM: 670nm) to track their distribution in Balb-c nude mice. Fluorescence imaging of Balb-c nude mice injected with AuNPs@(*m*PEG-PLGTs)-H and AuNPs@(*m*PEG-PLGTs)-OH was performed at 2h, 12h, 24h, and 48h post the second injection.

10. Histology analysis

Following the completion of animal experiments, female Balb-c mice were euthanized to retrieve major organs (heart, liver, spleen, lung, and kidney), which were fixed in 4% paraformaldehyde. Subsequently, the fixed specimens underwent a series of procedures including cleaning, dehydration, and embedding in paraffin. The slides were then immersed in Harris hematoxylin solution, washed, and transferred to eosin solution. After a final rinse in deionized water, dehydration was carried out using a gradient of ethanol concentrations, followed by immersion of the slides in a clearing

agent. Finally, the slides were sealed with mounting medium and prepared for observation. Tissue structure images were captured using an imaging system.

11. ^{1}H NMR Spectrum of AA-NCA and *m*PEG-PLGTs

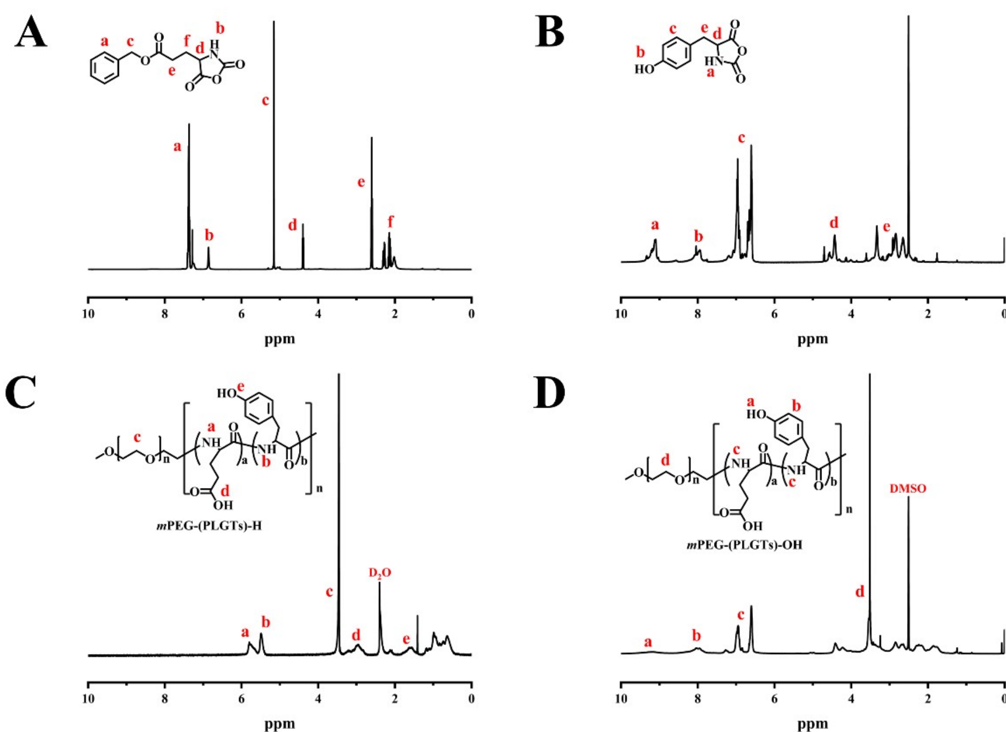

Fig. S2: A：^{1}H NMR Spectrum of Bn-L-Glu-NCA, B：^{1}H NMR Spectrum of L-Tyr-NCA, C：^{1}H NMR Spectrum of (*m*PEG-PLGTs)-H, C：^{1}H NMR Spectrum of (*m*PEG-PLGTs)-OH

12. Molecular weight of *m*PEG-PLGTs

Table S1.

Polymer	RT/min	Mn/kDa	Mw/kDa	Mv/kDa	PDI
(*m*PEG-PLGTs)-H	8.899	26.563	31.150	30.535	1.173

(*m*PEG-PLGTs)-OH	9.379	17.741	20.021	19.686	1.128