Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Temperature/Near-Infrared-Responsive Conductive Hydrogel for Controlled Drug Release and Real-Time Monitoring

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1. Experimental Section

1.1 Materials

N-Isopropylacrylamide (99%) was purchased from J&K Chemicals (Beijing, China). Ammonium persulfate (APS) and polylactic acid (PLA) was purchased from Aladdin Industrial Corporation (Shanghai, China). Dopamine hydrochloride, *N*,*N*′-Methylenebisacrylamide (BIS) and tetramethylethylenediamine (TMEDA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Graphite oxide (GO, diameter: 0.1–5 μm) was purchased from Nanjing JI Cang Nano Technology Co. Ltd. (Nanjing, China) and epidermal growth factor (EGF) was purchased from Beijing Biosynthesis Biotechnology Co. Ltd. (Beijing, China). The enzyme-linked immunosorbent assay kit was purchased from R&D Systems (Minneapolis, MN, USA). Fetal bovine serum (FBS), phosphate buffer solution (PBS, pH 7.4), Dulbecco's modified Eagle's medium (DMEM), and 1% penicillin–streptomycin solution were purchased from Corning (Corning, NY, USA). All other reagents and solutions were reagent grade and used as received.

1.2 Preparation of PDA-NPs

The typical oxidation and self-polymerization procedure used to synthesis PDA-NPs has been described in previous reports.[1] Briefly, NH_4OH (3 mL, 28%–30%) was added to ethanol (80 mL) and deionized water (180 mL), and the mixture was stirred at 25 °C for 30 min. Then, dopamine hydrochloride powder (0.5 g) dissolved in deionized water (20 mL) was poured into the above mixture, and polymerization was allowed to proceed under gentle magnetic stirring at 25 °C. After 30 h, the PDA-NPs were isolated by centrifugation at 11000 rpm for 10 min and washing with water three times. After lyophilization, a PDA-NPs powder was obtained. The morphology of the PDA-NPs was characterized by SEM (Nova 200 Nanolab, FEI Instruments) and transmission electron microscopy (TEM, Tecnai G2 20 S-TWIN, FEI Instruments). The synthesized PDA-NPs were found to be spherical, with an average diameter of ~200 nm and a uniform size distribution.

1.3 Preparation of Graphene Aerogel

The graphene aerogel was prepared by a reported method.[2] Briefly, GO powder (7.5 mg) and ascorbic acid (15 mg) were dissolved in deionized water (3 mL) in a sealed cylindrical glass vial and then immersed in a boiling water bath for 15 min to obtain a partially reduced GO dispersion. Next, the vial was placed in a -80 °C freezer and frozen for 0.5 h. After thawing at room temperature, the vial was again immersed in a boiling water bath to complete the reduction process. After 5 h, the reaction mixture was freeze-dried to obtain the graphene aerogel.

1.4 Preparation of PNIPAM-GO/PDA-NPs Hydrogels

The PNIPAM-GO/PDA-NPs hydrogels were prepared by in situ polymerization of PNIPAM within the graphene aerogel. In a typical synthesis procedure, a mixture of *N*-

isopropylacrylamide (1.13 g), BIS (cross-linker, 115 μL) , APS (initiator, 22.6 mg), and PDA-NPs (10 mg) was dissolved in deionized water (10 mL) and then purged with nitrogen for 20 min to remove oxygen. Subsequently, the dispersion was mixed with TMEDA (10 μL) and introduced into the graphene aerogel via vacuum-assisted backfilling. Finally, polymerization at room temperature for 12 h gave the PNIPAM-GO/PDA-NPs hydrogels.

1.5 Microscopic Morphology of Hydrogels

The morphologies of the PNIPAM, PNIPAM/PDA-NPs, PNIPAM-GO, and PNIPAM-GO/PDA-NPs hydrogels were characterized using SEM. Before observation, the hydrogels were freeze-dried for 3 days and sputter-coated with a layer of gold.

1.6 Mechanical and Adhesiveness Tests

The mechanical performance of the hydrogels was evaluated at room temperature using a rheometer (Z010TE, Zwick/Roell) equipped with parallel plates in the frequency sweep mode. The tensile strength was measured using a universal testing machine (ISO 1798, Instron) at a velocity of 100 mm/min. All the tested samples were in the form of rods with a diameter of 4.5 mm and an initial tensile length of 12 mm. The adhesive strengths of the hydrogels were also determined using the universal testing machine, and the crosshead speed was conducted at 100 mm/min. Mouse skin was used as the represented skin tissue. The hydrogels were adhered to the mouse skin with a bonded area of 20 mm × 15 mm. The adhesion strength was calculated by the measured maximum force divided by the bonded area.

1.7 Hydrogel Swelling and Deswelling Behavior

The hydrogel swelling and deswelling behavior was observed by immersion in water at 25 and 50 °C, and the swelling ratios of the hydrogels were determined. First, each sample (pure PNIPAM, PNIPAM/PDA-NPs, PNIPAM-GO, and PNIPAM-GO/PDA-NPs hydrogels) was freeze-dried and then the initial weight (W_i) was recorded. Subsequently, the dried samples were immersed in deionized water for 3 days until the equilibrium state was reached. After removing excess surface water, the final weight (W_f) of each swelled hydrogel was recorded. The hydrogel swelling ratio was calculated using the following equation:

$$\text{Swelling ratio (\%)} = (W_f - W_i)/W_i \times 100\% \quad (1)$$

1.8 NIR Response

The response characteristics of the hydrogels to NIR irradiation were investigated using an NIR laser (808 nm) with a power density of 3.15 W/cm^2. The volume change of each hydrogel (PNIPAM, PNIPAM/PDA-NPs, PNIPAM-GO, and PNIPAM-GO/PDA-NPs hydrogels) was quantified by measuring the size of a cylindrical hydrogel sample before and after irradiation with an NIR laser for 3 min. The initial cylindrical samples had a height of 5 mm and a diameter of 9 mm. The deswelling ratio

of each hydrogel was expressed as the shrinkage volume ratio (V_t/V_0, where V_t and V_0 are the volumes of the hydrogel sample after irradiation and before irradiation, respectively). To investigate the thermal reversibility following NIR irradiation, the surface temperatures of the hydrogel samples were monitored using an NIR temperature detector during on–off cycling of the laser (irradiation with the NIR laser for 1 min followed by cooling in water at room temperature for 30 min). The photothermal efficiencies of the hydrogels were evaluated by NIR irradiation for 6 min. A rectangular hydrogel strip (30 mm × 5 mm × 3 mm) was used to detect the bending deformation ability of the hydrogel for potential actuator applications.

1.9 Drug Release Behavior Under NIR Irradiation

The NIR-irradiation-induced drug release behavior of the PNIPAM-GO/PDA-NPs hydrogel was investigated using DOX as a model drug. The DOX-loaded hydrogels were prepared by immersing the lyophilized hydrogel in a DOX solution (10 mg/mL) for 3 days until the hydrogel reached equilibrium. To assess the drug release capacity, the hydrogels were placed in PBS and subjected to NIR irradiation for 1 min after 15 min and after 30 min. The solution was collected and replaced with an equal amount of fresh PBS at predetermined intervals. Then, the amount of released DOX was monitored using an ultraviolet-visible-NIR spectrophotometer (Lambda 950, PerkinElmer). The drug release behavior of the hydrogel in the air was tested under a laser on-off cycle (on: 1min, off: 5min), and the released DOX or EGF (EGF-loaded hydrogel was prepared using the same procedure that prepared DOX-loaded hydrogels) was collected for monitoring. At the same time, the resistance of the hydrogel was recorded by an avometer.

1.10 Simulated drug release and real-time monitoring

To simulate the release behavior of the drug from the hydrogel patch on a mouse wound, a creative device was fabricated by 3D printing technology using PLA. The device is similar to a tea cup, with the top and bottom separated by a permeable membrane. The lower half of the device was filled with PBS and placed in an environment with the temperature of 37 °C. The EGF-loaded hydrogel (prepared using the same procedure that prepared DOX-loaded hydrogels) was placed in the upper half of the device and connected to the avometer. The solution in the lower half of the device was collected and replaced with an equal amount of PBS at predetermined intervals. The amount of released EGF was monitored by ELISA kit (R&D Systems). At the same time, the resistance of the hydrogel was recorded. Further verification experiments were performed using the same method at different time points.

1.11 *In Vitro* EGF Loading and Release

EGF-loaded hydrogels were prepared using the same procedure that prepared DOX-loaded hydrogels. The tested hydrogels including PNIPAM hydrogels, PNIPAM-GO hydrogels, PNIPAM/PDA-NPs hydrogels and PNIPAM-GO/PDA-NPs hydrogels. The *in vitro* release of EGF from the hydrogels was monitored in the PBS at 37 °C. At

predetermined intervals (1, 3, 5, 10, and 15 days), the release medium was collected, and an equal amount of fresh PBS was added to the release medium. The amount of released EGF in the collected release medium was measured by an ELISA kit.

1.12 Cell affinity

The cell affinity was tested by the hydrogels of PNIPAM, PNIPAM-GO, PNIPAM/PDA-NPs and PNIPAM-GO/PDA-NPs. Before cell culture, the hydrogels were purified in PBS for 3 days and sterilized in 75 wt % alcohol. Melanoma cells (B16F10) were seeded onto the hydrogels with a density of 1×10^4 cells/ml. The cell growth medium was composed of DMEM supplemented with 10% FBS and 1% penicillin−streptomycin. The cells were maintained in a 5% CO_2 incubator at 37 °C. The CCK-8 kit was used to evaluate the proliferation of the cells after 1 and 2 days of culture. The fluorescence microscopy of cells attached on the hydrogels was observed using a confocal laser scanning microscope (UltraVIEW VoX, PerkinElmer).

1.13 *In Vivo* Wound Healing

The wound healing ability of the hydrogels was investigated using six male Sprague Dawley (SD) rats weighing 180–200 g each. First, the rats were anesthetized with pentobarbital (2 wt %, 2 mL/kg) and the backs were depilated. Then, three circular wounds were created on each rat using a biopsy punch. One of the wounds was used as a control and the other two were treated with the PNIPAM-GO/PDA-NPs hydrogel and an EGF-loaded PNIPAM-GO/PDA-NPs hydrogel (EGF, 30 μg/sample). Subsequently, the wound healing performance of the rats, recorded using a digital camera at predetermined intervals (0, 5, 9, and 14 days), was evaluated based on wound size reduction measurements. On day 15, the regenerated skin at the wound site was harvested and immersed in 4% buffered paraformaldehyde. Finally, the samples were stained with Hematoxylin–eosin and Masson's trichrome stain for histological observations.

2. Supplementary Figures

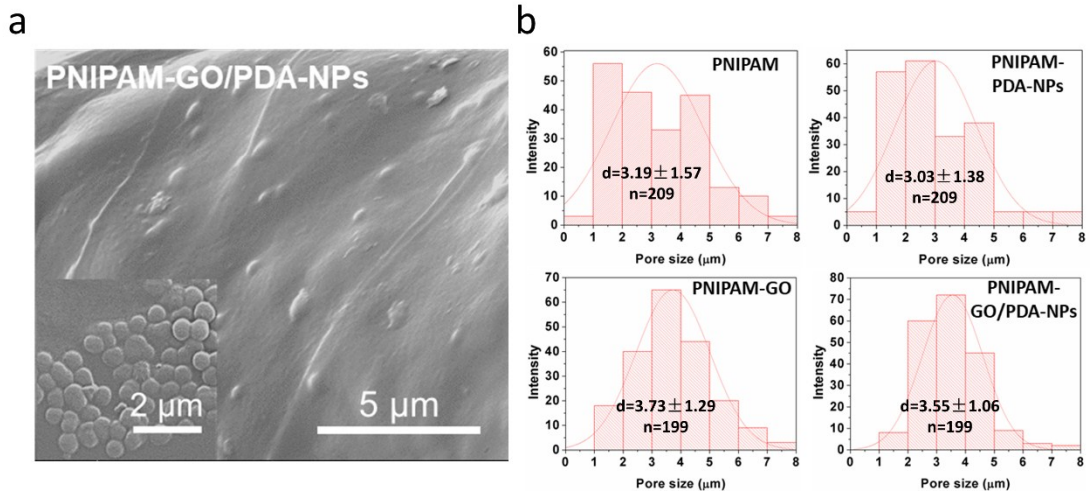

Figure S1. (a) SEM images PNIPAM-GO/PDA-NPs hydrogel. (b) Pore size distribution of PNIPAM, PNIPAM-GO, PNIPAM/PDA-NPs, and PNIPAM-GO/PDA-NPs hydrogels.

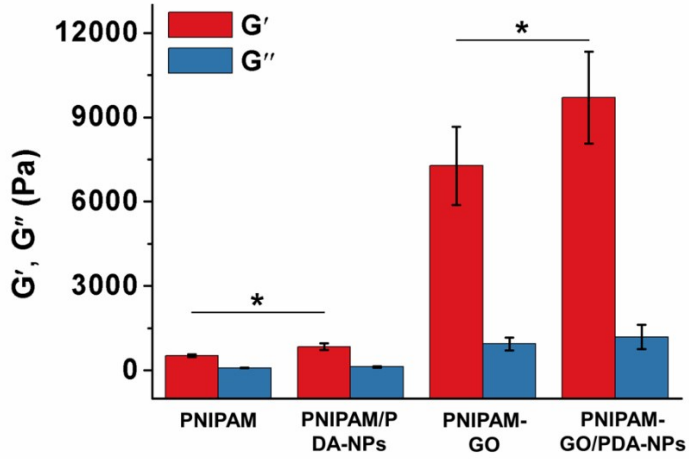

Figure S2. Storage (G′) and loss (G″) moduli of PNIPAM, PNIPAM-GO, PNIPAM/PDA-NPs, and PNIPAM-GO/PDA-NPs hydrogels. *$p < 0.05$.

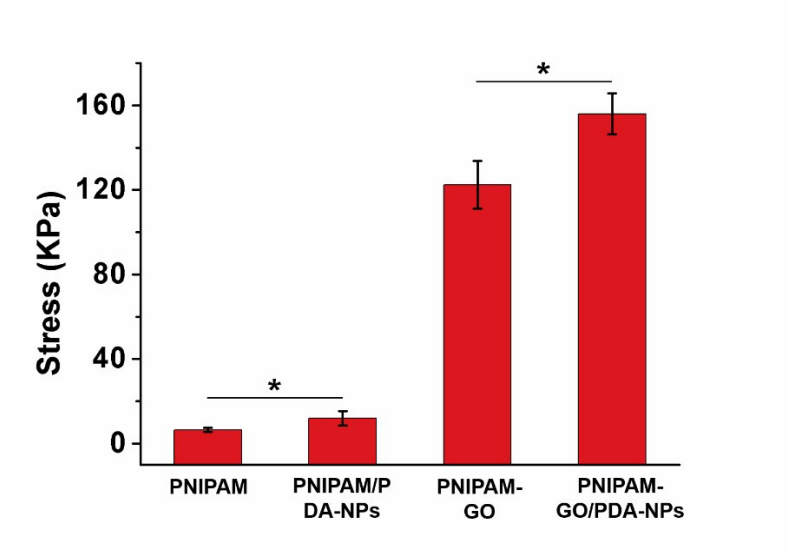

Figure S3. Tensile stress of PNIPAM, PNIPAM-GO, PNIPAM/PDA-NPs, and PNIPAM-GO/PDA-NPs hydrogels. *$p < 0.05$.

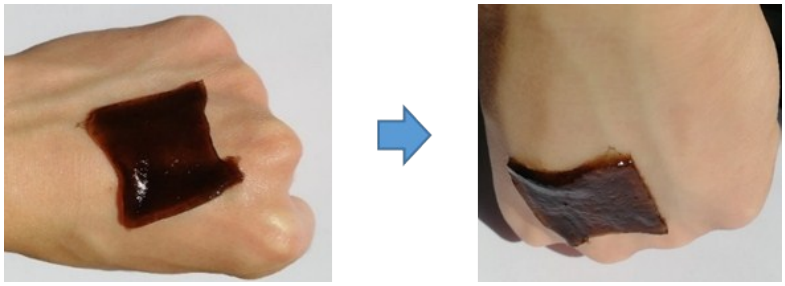

Figure S4. Adhesion of the PNIPAM-GO/PDA-NPs hydrogel to skin on the hand.

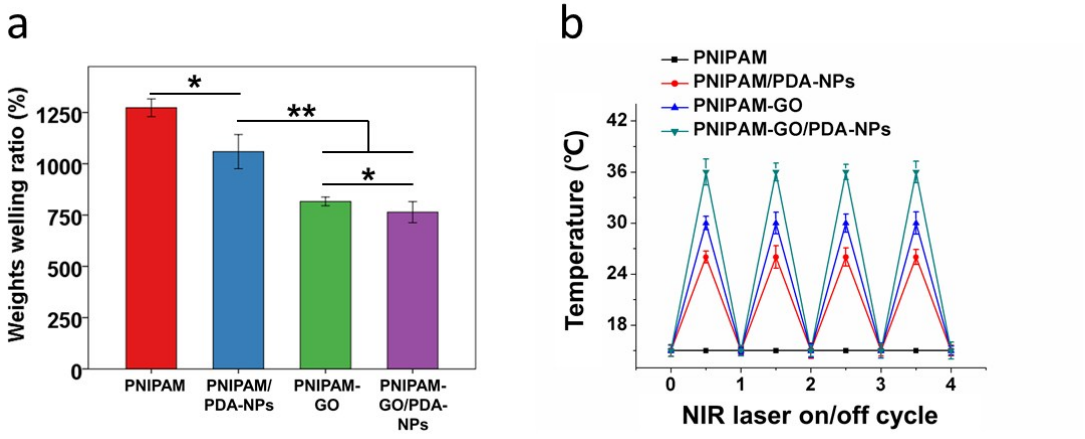

Figure S5. (a) Equilibrium swelling weight ratios of PNIPAM, PNIPAM-GO, PNIPAM/PDA-NPs, and PNIPAM-GO/PDA-NPs hydrogels at room temperature. (b) Temperature changes of PNIPAM, PNIPAM-GO, PNIPAM/PDA-NPs, and PNIPAM-GO/PDA-NPs hydrogels as a function of NIR irradiation-cooling cycles (irradiation time: 1 min). *$p < 0.05$, **$p < 0.01$.

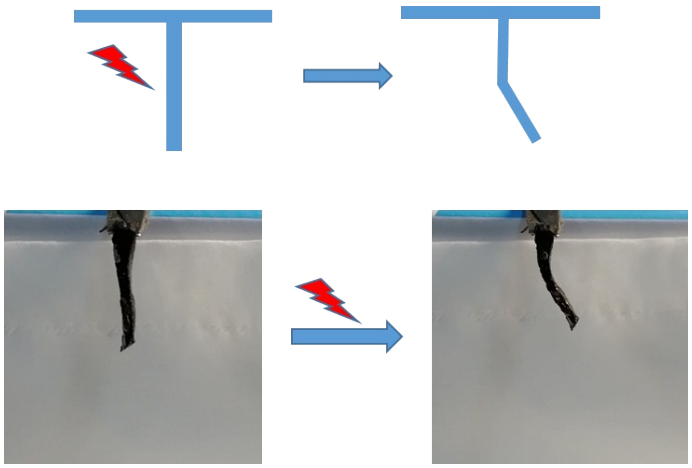

Figure S6. Photographs of the PNIPAM-GO/PDA-NPs hydrogel before and after NIR irradiation (power density: 3.15 W/cm^2, irradiation time: 10 s).

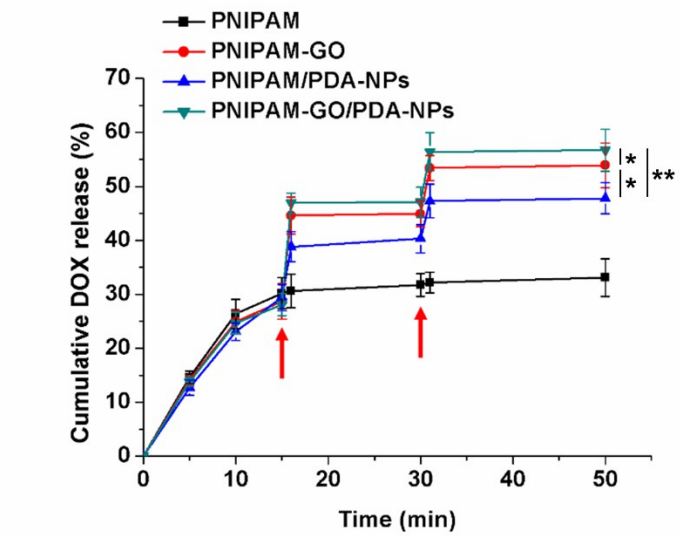

Figure S7. Cumulative DOX release profile of dexamethasone from PNIPAM, PNIPAM-GO, PNIPAM/PDA-NPs, and PNIPAM-GO/PDA-NPs hydrogels without NIR irradiation and in response to NIR laser irradiation for 1 min (red arrows, power density: 3.15 W/cm^2). *$p < 0.05$ and **$p < 0.01$.

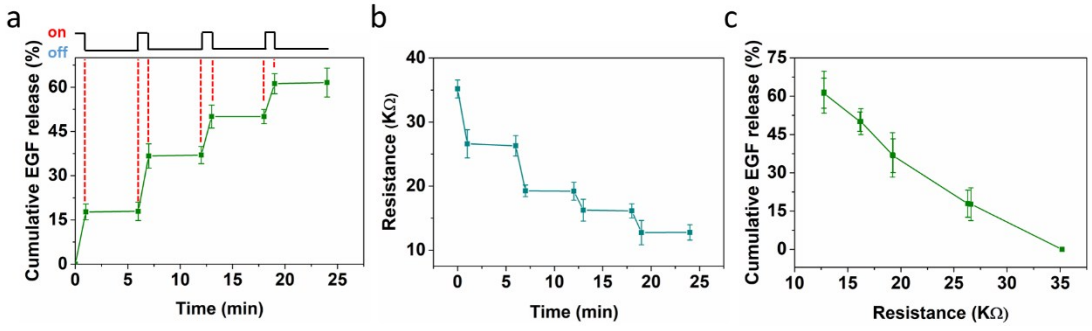

Figure S8. NIR-light-triggered (a) drug release from the PNIPAM-GO/PDA-NPs hydrogel and (b) resistance change of the PNIPAM-GO/PDA-NPs hydrogel. The hydrogel was intermittently irradiated with NIR light at 3.15 W/cm^2, each time for 1 min. (c) Correlation between controlled drug release from the PNIPAM-GO/PDA-NPs hydrogel by NIR irradiation and the resistance of the hydrogel.

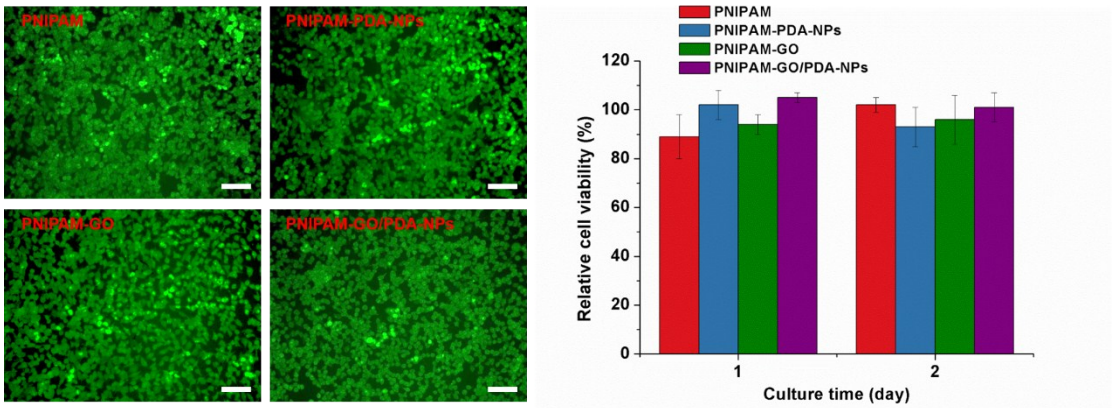

Figure S9. Fluorescence microscopy images and relative viability of B16-F10 cells incubated with PNIPAM, PNIPAM-GO, PNIPAM/PDA-NPs, and PNIPAM-GO/PDA-NPs hydrogels. Scale bar: 100 μm.

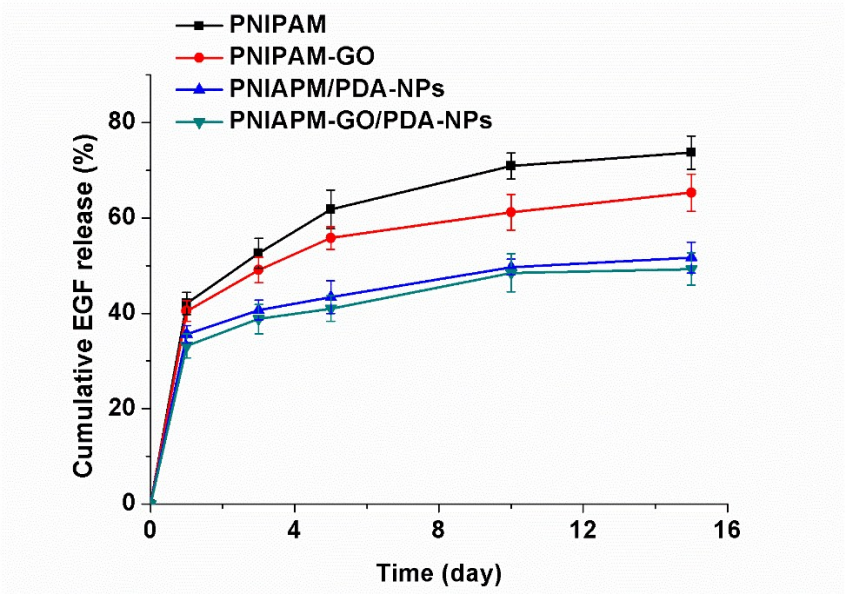

Figure S10. EGF release from the PNIPAM, PNIPAM-GO, PNIPAM/PDA-NPs, and PNIPAM-GO/PDA-NPs hydrogels.

3. References

(1). K. Ai , Y. L. Liu , C. P. Ruan, L. H. Lu, G. Q. Lu, *Adv. Mater.*, 2013, **25**, 998.
(2). L. Qiu, J. Z. Liu, S. L. Y. Chang, Y. Wu, D. Li, *Nat. Commun.*, 2012, **3**, 1241.