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Supporting information for

Surface-engineered NIR light-responsive actuator for controllable modulation of collective cell migration

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Experimental Section

1. Materials

N-Isopropylacrylamide (NIPAM), Acrylamide (AM), Fluorescamine, N, N'-Methylenebisacrylamide (MBA), N-(3-Aminopropyl)methacrylamide hydrochloride (APMA), Hexadecyl trimethyl ammonium bromide (CTAB) were all obtained from Aladdin (Shanghai, China). N,N'-Bis(acryloyl)cystamine (BAC), Triton™ X-100, Tris(2-carboxyethyl)phosphine (TCEP), N,N,N',N'-Tetramethylethylenediamine (TEMED), (3-Aminopropyl)trimethoxysilane, $AgNO_3$, Bovine serum albumin (BSA) Anti-Rabbit IgG (H+L) tagged with CF™ 568 antibody produced in goat (SAB4600085), Anti-Paxillin antibody produced in rabbit (SAB4502553), Fluorescein Isothiocyanate Labeled Phalloidin (P5282) were purchased from Sigma-Aldrich. Trisodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), Ammonium persulphate (APS), $NaHCO_3$, Maleic anhydride (MAH), Glutaraldehyde (25%), Ascorbic acid, $NaBH_4$, Ethanol, $HAuCl_4 \cdot 4H_2O$, NaH_2PO_4, Na_2PO_4 were purchased from Sinopharm (Shanghai, China). Dulbecco's modified eagle medium (DMEM), Dulbecco's Phosphate-Buffered Saline (DPBS), fetal bovine serum, penicillin/streptomycin, Trypsin-EDTA (0.25%), were all obtained from Gibco (Thermo Fisher, U.S.A.). Methoxy-PEG-Lipoic acid (LA-PEG) was purchased from Shanghai ToYongBio Tech. Inc. (Shanghai, China). Cyclic Arg-Gly-Asp-D-Phe-Lys-(Cys) (cRGD) peptdies, Rhodamine B-labeled cRGD were purchased from Shanghai Apeptide Co., Ltd. (Shanghai, China).

2. Preparation and characterizations of the prepared AuNRs

The gold nanorods (AuNRs) were synthesized by using a seed-mediated growth method as described elsewhere.[1] For the preparation of gold seeds, 40.5 μL of 24.28 mM $HAuCl_4$ was firstly mixed with 4 mL of 0.1 M CTAB solution, followed by the addition of 24 μL of 0.1 M $NaBH_4$ under stirring. Then the gold seed solution was kept undisturbed for 2 h before use. To prepared the AuNR growth solution, 2 mL of 0.01 M $HAuCl_4$ and 0.4 mL of 0.01 M $AgNO_3$ were injected into 40 mL of 0.1 M CTAB, followed by the addition of 80 μL of HCl (37 wt%). 0.32 mL of 0.1 M ascorbic acid was added and the solution under stirring. For the synthesis of 808 nm AuNRs, 96 μL of seed solution was added into the growth solution. After stirred for 60 s, the final mixture was kept rest overnight. The prepared AuNRs were characterized by UV-visible spectroscopy (Shimadzu UV-1800, Japan) and TEM (JEM 1230, JEOL, Japan).

3. Functionalization of AuNRs

For the functionalization of AuNRs, 10 mL of 0. 4 mM BAC in ethanol was mixed with 30 ml of AuNR solution under vigorously stirring at 600 rpm overnight. After the completed ligand exchange, the

residual ligands was removed by centrifugation at 8,000 rpm for 10 min for three times. The functionalized AuNRs were redispersed in 6 mL water.

4. Surface modification on glass substrate

To obtain amine modified glass substrates, cleaned coverslips were immersed into the solution of 5% (3-aminopropyl) trimethoxysilane (APTES) in ethanol for 12 h. Then the coverslips were thoroughly washed with ethanol and water for three times, respectively. After dried under N_2 flow, the amine modified glass substrates were placed in a glass dish, followed by the addition of 10 mL PB solution (pH=7.4) containing 5% glutaraldehyde. After the reaction was completed, the glass substrate was washed with water for three times and dried before use.

5. Preparation of copolymer hydrogel actuator

The copolymer hydrogel was produced according to the previously reported protocol with slight modification.[2] For preparing the copolymer hydrogel with 95% NIPAM in total monomer, 26.4 μL of NIPAM (1.593 M), 1.4 μL of AM (2.535 M), 11 μL of MBA (0.097 M) and 6 μL of APS (0.070 M) were mixed in a 200 μL centrifuge tube, followed by the addition of 15 μL of AuNRs and 0.5 μL of TEMED, respectively. The mixture was transformed onto the surface-modified glass substrate, on which 5.3 μL of APMA (0.056 M) was added in advance. Then the reaction solution was quickly coated with another cleaned coverslip. After the polymerization reaction was completed, the coverslip was slowly removed from the formed copolymer hydrogel. To prepare the hydrogel with different thermal responses, the mass ratio of NIPAM to the total monomer (NIPAM and AM) was tuned from 60% to 100%. Moreover, poly(AM) hydrogel doped with gold nanorods was prepared with the same procedures for investigating the effect of temperature on cell mobility in our experiments.

Meanwhile, AuNRs with different concentrations (at ~200 pM, ~400 pM and ~800 pM, respectively) was used to make the hydrogel with optimized NIR light responses. The AuNRs in the prepared hydrogel were detected with a darkfield microscope (Nikon 80i, Japan), which equipped with a color CCD camera (Olympus DP72, Japan). The residual primary amino group on hydrogel surface was detected with the fluorescamine assay with a reversed fluorescence microscope (ZEISS Axio Vert A1, Germany).

6. Investigating the responses of the hydrogel actuator

To investigate the NIR light-stimulated mechanical actuation, the responses of hydrogel actuator was firstly characterized in vitro. By irradiating the hydrogel actuator with a commercial NIR laser (808nm, K808D02FN-8.000W, BWT) at different intensity from 0 to 24 mW/mm^2, the changes in temperature for

the hydrogel were recorded with a thermal imager (FLIR-E64501, FLIR). The time course of temperature increase of the hydrogel actuator was then analyzed by plotting the temperature at different time and at different irradiation intensity. We also examined the reversibility of hydrogel temperature elevation and descent by switching on and switching off the laser alternately. Meanwhile, we investigated the thermal responses of the hydrogel actuator by recording the contraction of a round shape hydrogel actuator placed in a water-bath at different temperature. The changes in hydrogel contraction was record by a digital camera and analyzed by ImageJ. For the optimization of NIPAM ratio and AuNRs concentration for hydrogel preparation, the NIR light irradiation induced temperature elevations were also obtained with the same procedures. To examine the morphology change of the hydrogel actuator, the prepared hydrogels after separately immersing in the water-bath at 25 °C and 40 °C were quickly frozen at -80°C, followed by vacuum freeze-drying for 16 h. Then the morphology of the hydrogel was characterized with a scanning electron microscope (JSM-6700F SEM).

The mechanical properties of the hydrogel actuator were investigated by using a previously reported method. After immersing in the water-bath at different temperature and removing residual water on the gel surface , the hydrogels were then weighted and the water content (ω) was calculated according to the equation,[3] $\omega = (m_1 - m_0)/m_1$, in which m_0 and m_1 represent the weight of dried hydrogel and water-swollen hydrogel, respectively. Then the mechanical property of the hydrogel actuator was estimated based on the established correlation between Yong's modulus and water content,[4] which is given by

$$E = 351075 \times (1 - 0.8982\omega)e^{-(1.0444-\omega)^{4.15} \cdot (log(1/d))^{7.15\omega^{2.79}} + 1.55\omega^{17.41}}$$

where E represents the Yong's modulus, ω and d are the water content and molar ratio of cross-linker/monomer in the hydrogel, respectively. The effect of temperature elevation on mechanical property of the hydrogel actuator was obtained by plotting the Yong's modulus as a function of temperature.

7. Surface functionalization of OMAC hydrogels

To make the hydrogel actuator compatible for cell culture, the cRGDfk peptides were conjugated onto the hydrogel surface.[5] Firstly, hydrogel was first immersed into 2 mL of 100 mM $NaHCO_3$ solution, and then 400 μL of 0.5 M maleic anhydride in ethanol was added for a incubation of 2 h. After thoroughly washed with water, the hydrogel was incubated with 1 mL of cRGDfk peptide solution (1 mM). The functionalized hydrogel actuator was thoroughly washed with water before use. To facilitate the characterization of RGD peptides on the surface of hydrogel actuator, the cRGDfk peptides were replaced by the fluorescently labeled RGD peptides.

8. Patterned modification of RGD peptides with a microcontact printing method

To present the RGD peptides with specific patterns, the microcontact printing method was applied during the processes of RGD peptide grafting.[6, 7] Briefly, after the primary amino group on the surface of hydrogel actuator was crosslinked with maleic anhydride, the hydrogel actuator-attached glass substrate was reversely coated onto a microgrooved PDMS stamp. Then 60 μL of cRGD peptide solution (10 mM) was introduced into the microgrooves via capillary action. After removing residual cRGD peptides by washing the hydrogel, 25 μL of 2.5 mM PEG-DHLA (obtained by reducing PEG-LA with TCEP) solution were used to block the residual active sites on the hydrogel surface. The residual reagents on the surface of hydrogel actuator were remove by thoroughly washing with water.

9. Cell culture and cell imaging

Human hepatocellular carcinoma (HepG2) cell line was obtained from the American Type Culture Collection (ATCC, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), and 1% streptomycin/ penicillin. The cells were kept at 37 °C with 5% CO_2 in a humidified atmosphere. After washing the hydrogel actuator with PBS, the cells were seeded on hydrogel actuator. For the investigations of cell adhesion and cell proliferation, the number of cells after the incubation of different time (at 4h, 8h, 12 h and 48 h, respectively) were counted. The NIR light actuation of cell behaviors was conduct by irradiating the cells culture on the hydrogel actuator with the 808 nm light with different intensity from 0 to 24 mW/mm^2, the responses of cell behaviors were detected under bright-field microscopy.

To achieve continuous activation of the optomechanical actuator, the alternating light irradiation manner was adopted based on their thermal responses under NIR light stimulation, where the irradiation time and rest time for single period of cell actuation were assigned as 54 s and 6 s, respectively. To prove the influence of NIR light on cell behaviors, cells cultured both on glass substrate and poly(AM) hydrogel doped with AuNRs after NIR light irradiation at 24 mW/mm^2 was also investigated as the control experiment. To study cell viability without or with the NIR light irradiation at 24 mW/mm^2, the live-dead cell staining experiment (live-dead cell staining kit, BestBio) was performed by following the protocol of the commercial staining kit. The cell imaging was conducted under fluorescence microscope.

For studying the optical actuation induced mechanical signal transduction, we investigated the mechanical signaling-involved proteins by immunostaining. Firstly the changes of focal adhesion points in cells were investigated by immunostaining of paxillin. The cells cultured on hydrogel actuator with or

without NIR light irradiation at 24 mW/mm^2 were washed twice with PBS and fixed by adding 4% paraformaldehyde in PBS for 15min. After washed twice with PBS, the sample was incubated with 0.25% (v/v) Triton-X 100 dissolved in PBS for 10 min, and then washed twice with PBS to remove the detergent. For blocking non-specific adsorption, the sample was incubated with 1% (w/v) BSA in PBS containing 0.1 % (v/v) Triton-X 100 for 45 min. The cells were then incubated with the primary antibody-rabbit monoclonal to paxillin (SAB4502553, Sigma-Aldrich) diluted at a ratio of 1:200 (v/v) in PBS containing 0.1 % (v/v) Triton-X 100 for 1 h. After washing with PBS for three times, the sample was incubated with the anti-rabbit secondary antibody tagged with the fluorescent dye CF 568 (1:200 dilution, SAB4600085, Sigma-Aldrich). The imaging experiments were conduct under an inverted fluorescence microscope. To detect the changes of cellular actin cytoskeleton, the cells were fixed with 4% paraformaldehyde in PBS and then incubated with 0.25% (v/v) Triton-X 100 dissolved in PBS. To reduce non-specific adsorption, the sample was incubated with 1% (w/v) BSA in PBS containing 0.1 % (v/v) Triton-X 100 for 45 min. Finally, the sample was incubated in a solution of FITC labeled phalloidin (1:500 dilution, Sigma-Aldrich) in the dark for 1 h. After washing twice with PBS, the sample was observed with fluorescence microscopy.

10. **Image and data analysis**

All the images obtain in our work was analyzed with the ImageJ software, including the quantitative analysis of cell motility, the changes in cell outline as well as focal adhesion points. With the obtained cell trajectory, we calculated the velocity, travel distance and the cumulative distance for cells with or without NIR light irradiation. The shape index (SI) was calculated according to the equation,[8] $SI = E/2\sqrt{\pi A}$, where E represents the total length of cell contour boundary and A is the total area of the cell.

Reference

1 B. Xiong, R. Zhou, J. Hao, Y. Jia, Y. He and E. S. Yeung, *Nat.Commun.*, 2013, **4**, 1708.

2 T. Caykara, S. Kiper and G. Demirel, *Eur. Polym. J.*, 2006, **42**, 348-355.

3 M. Zhong, Y.-T. Liu, X.-Y. Liu, F.-K. Shi, L.-Q. Zhang, M.-F. Zhu and X.-M. Xie, *Soft Matter*, 2016, **12**, 5420-5428.

4 X. B. Zou, J. Bai, G. L. Yu, H. B. Wang and L. Yang, *Advanced Materials Research*, 2011, **233-235**, 1803-1808.

5 S. Tian, S. Zheng, Y. Han, Z. Guo, G. Zhai, X. Bai, X. He, E. Fan, Y. Zhang and K. Zhang, *Anal. Chem.*, 2017, **89**, 8259-8265.

6 S. Alom Ruiz and C. S. Chen, *Soft Matter*, 2007, **3**, 168-177.

7 A. P. Quist, E. Pavlovic and S. Oscarsson, *Anal. Bioanal. Chem.*, 2005, **381**, 591-600.

8 F. Lin, H. Zhang, J. Huang and C. Xiong, *ACS Biomaterials Science & Engineering*, 2018, **4**, 1337-1345.

Supplementary Figures

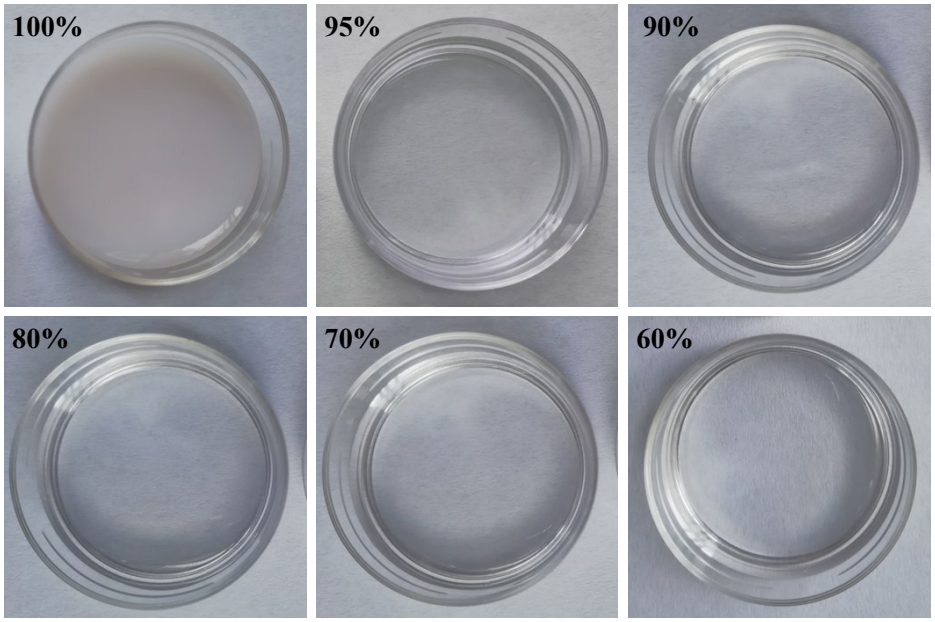

Figure S1. Analyzing the optical transparency of hydrogel actuators prepared with different NIPAM ration in the monomer from 100% to 60%.

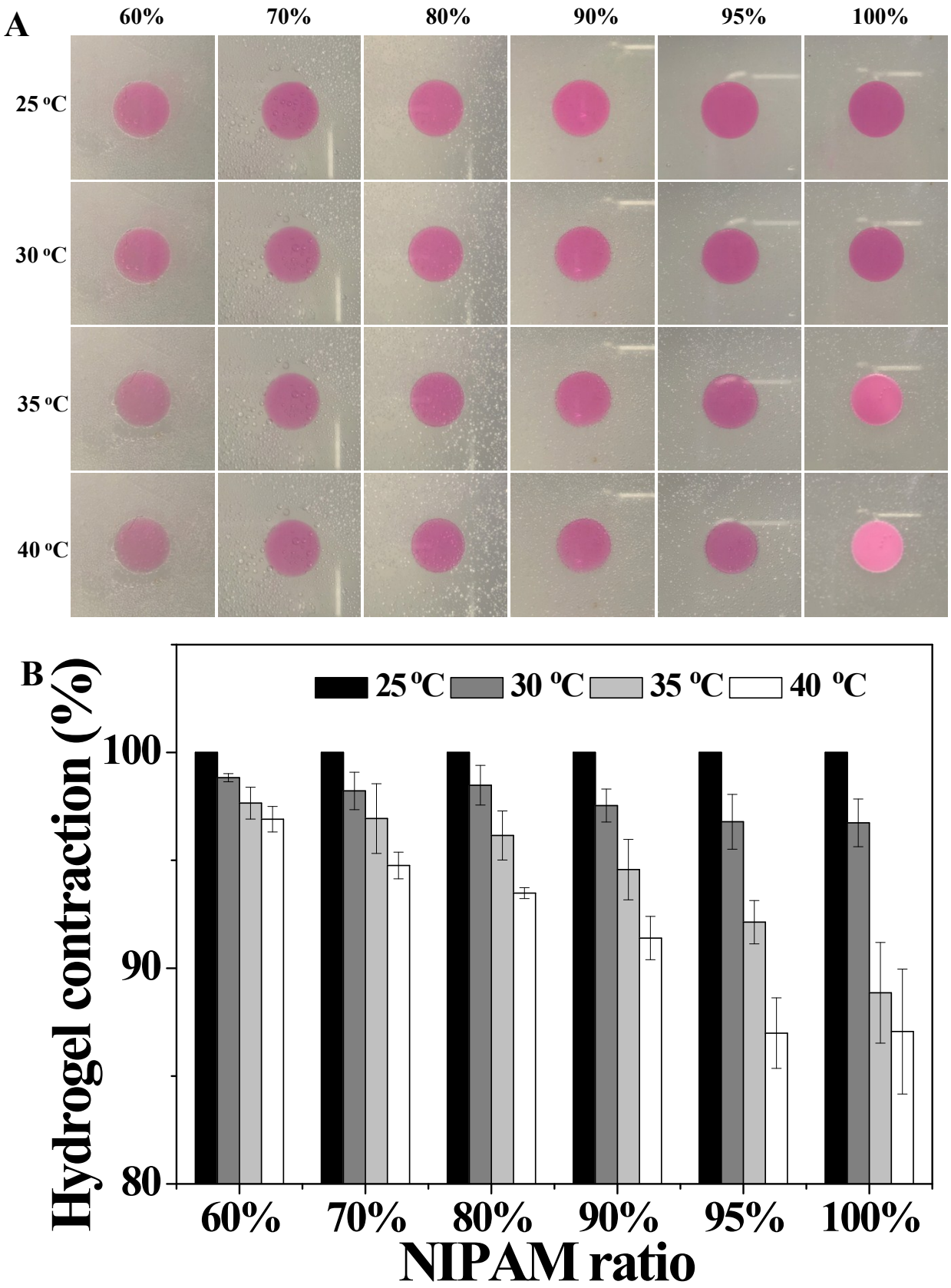

Figure S2. Characterizations of thermal responses for the hydrogel actuators. (A) Optical images of the hydrogel actuators. (B) Quantitative analysis the hydrogel contraction at different temperature.

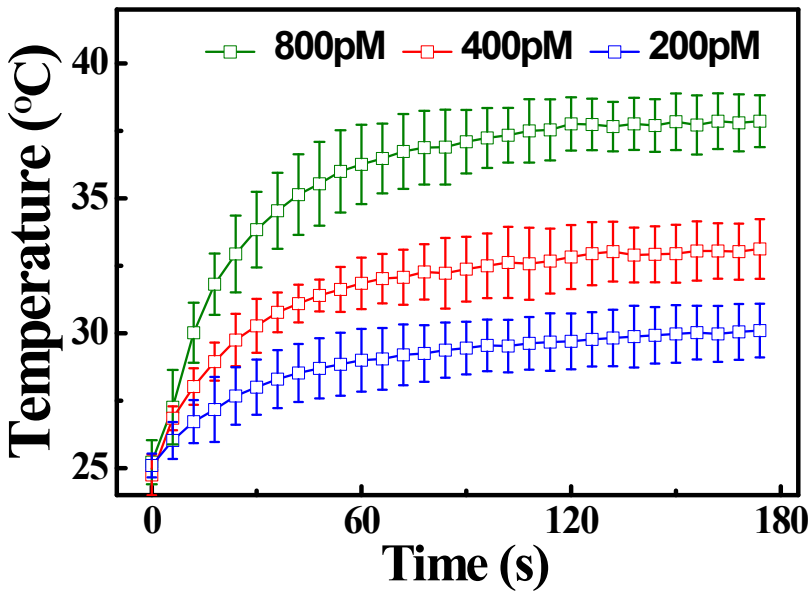

Figure S3. Optimizing the concentration of AuNRs used for the preparation of NIR light responsive hydrogel actuator.

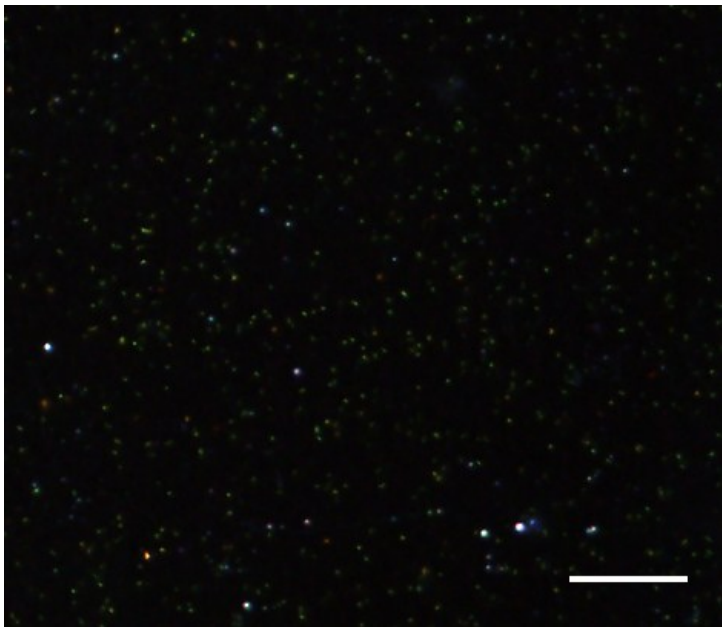

Figure S4. Darkfield imaging of AuNRs in solution. The Scale bar is 50 μm.

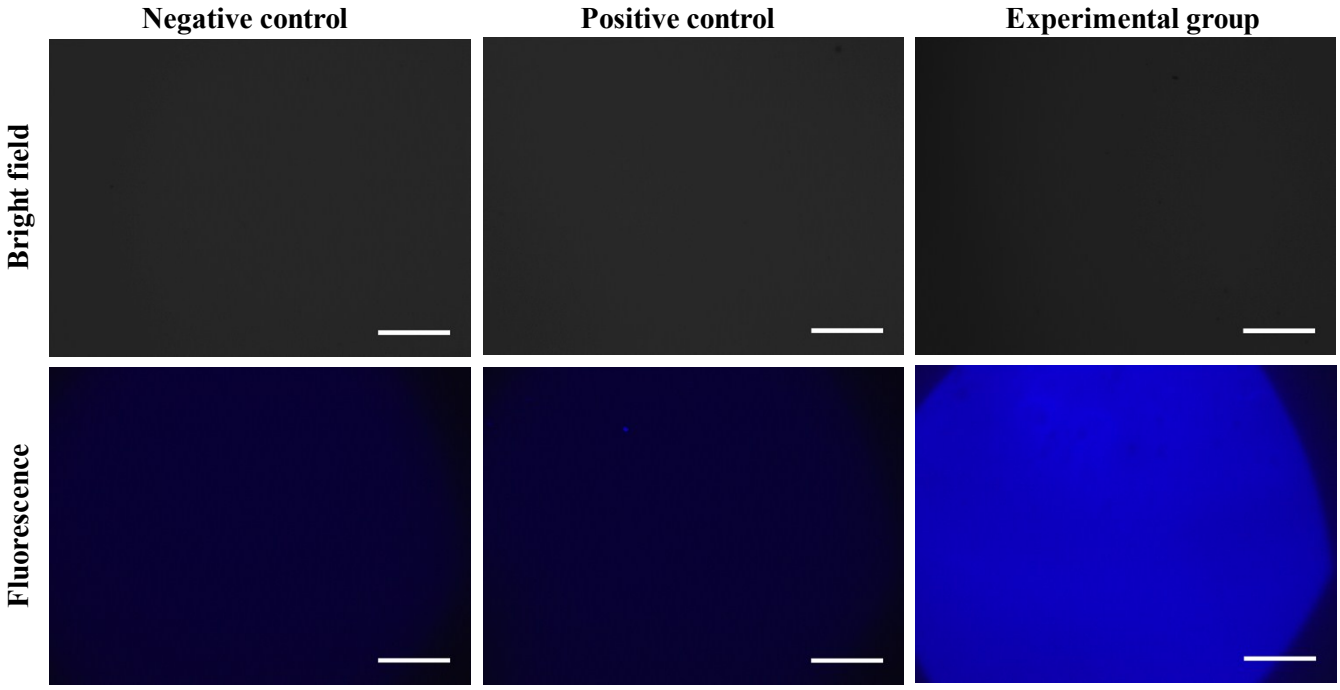

Figure S5. **Detecting the residual primary amine groups on the hydrogel actuator with the fluorescamine-based assay.** The hydrogel prepared without APMA (Negative control) and nonspecifically absorbed APMA (Positive control), or the hydrogel prepared with APMA (Experimental group) were investigated, suggesting the primary amine groups in AMPA molecules were successfully conjugated in hydrogel actuator. All scale bars equal 150 μm.

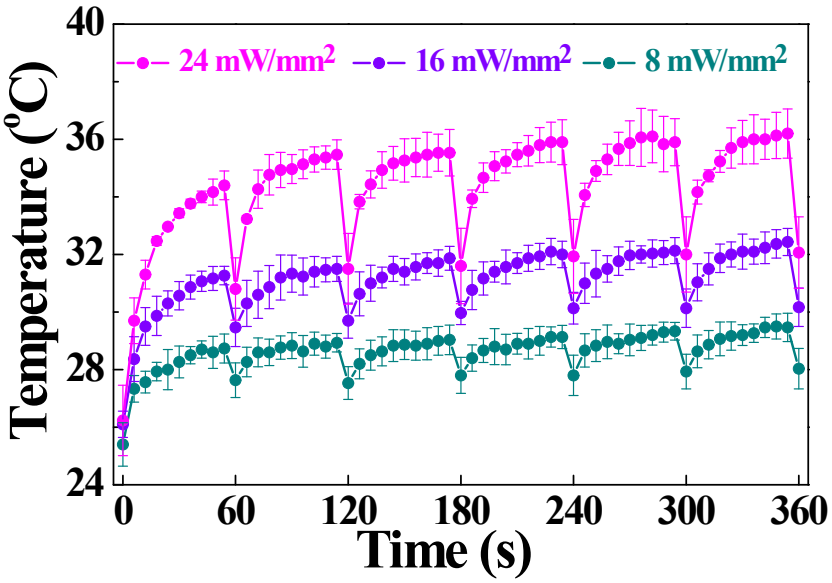

Figure S6. Changes of temperature for the hydrogel actuator under switchable irradiation of NIR light.

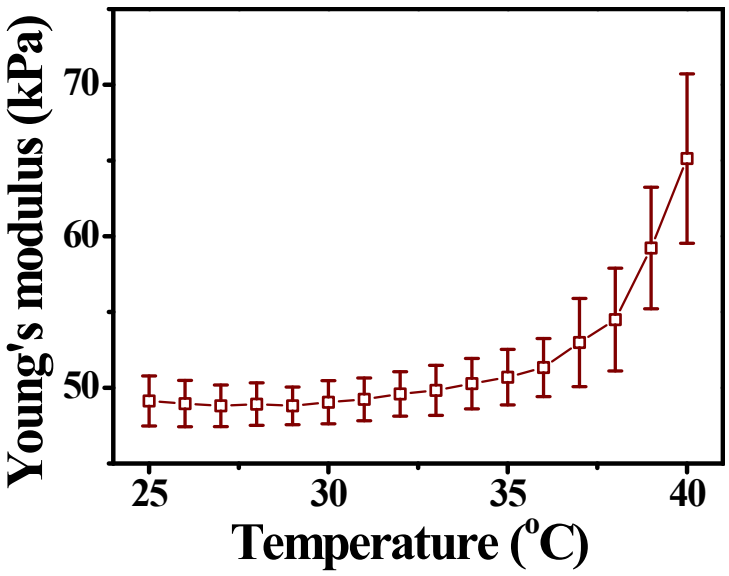

Figure S7. The Young's modulus of the prepared hydrogel actuator as a function of temperature.

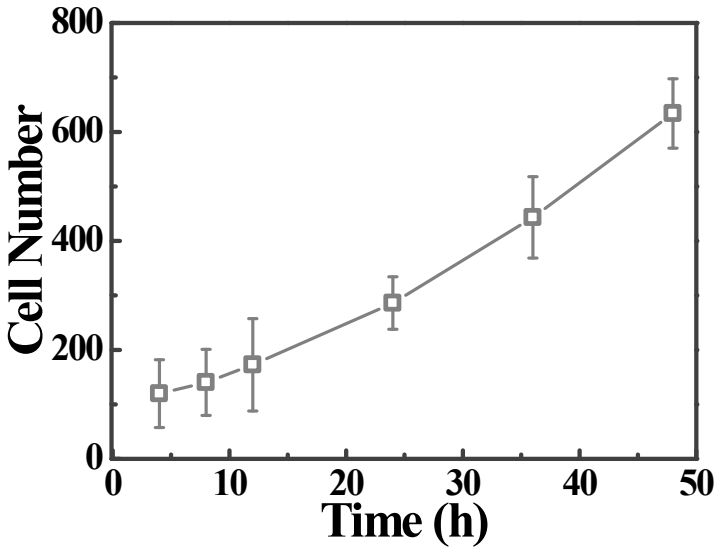

Figure S8. Quantitative analysis of cell proliferation on the hydrogel actuator.

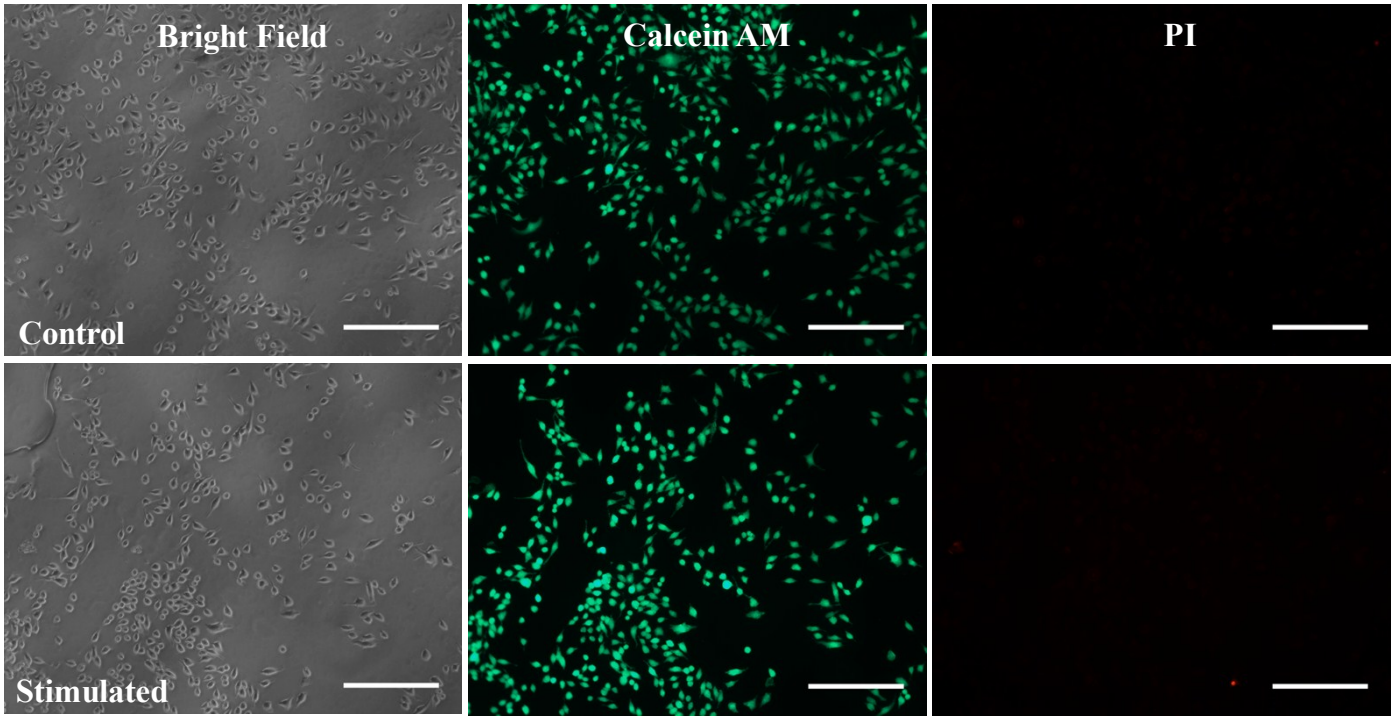

Figure S9. Investigating the influence of laser irradiation on cell viability without (Control) and with (Stimulated) NIR light irradiation at 24 mW/ mm^2. All scale bars equal 180 μm.

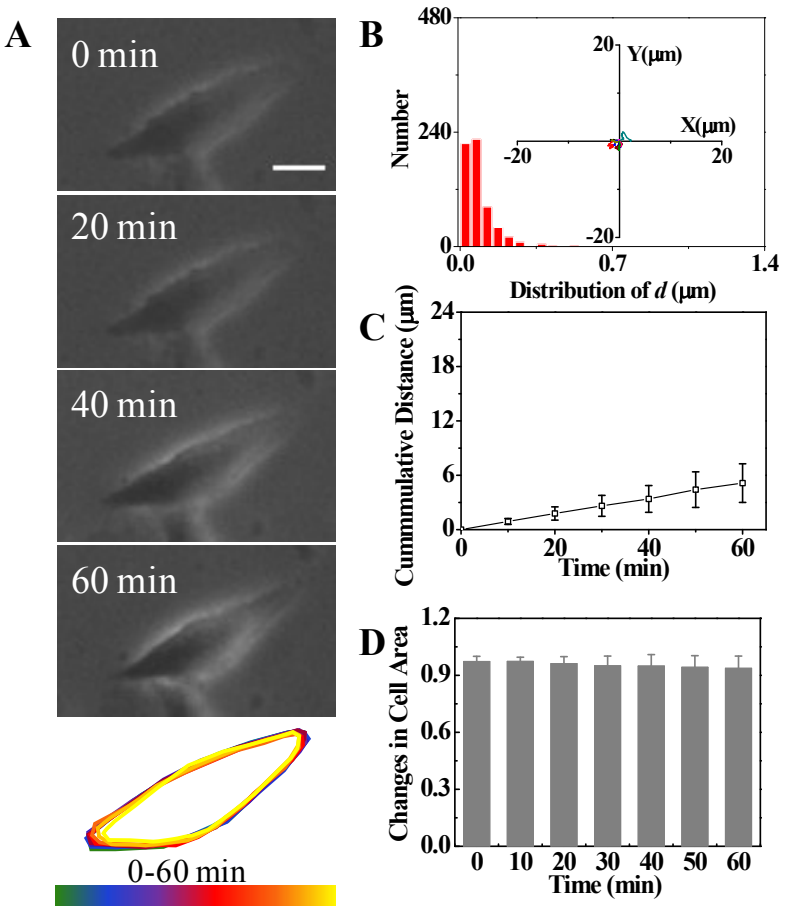

Figure S10. Investigating the effect of temperature on the migration behavior of cells cultured on poly(AM) hydrogel doped with AuNRs under NIR light irradiation. (A) Represent cell images under NIR light irradiation at 24 mW/mm^2. The changes in cell outline at different time was shown on the bottom. The scale bar is 15 μm. The travel distance distributions (C) and cumulative distance (D) for cells under stimulation with NIR light irradiation at 24 mW/mm^2. (D) The analysis of changes in cell area under NIR light stimulation.

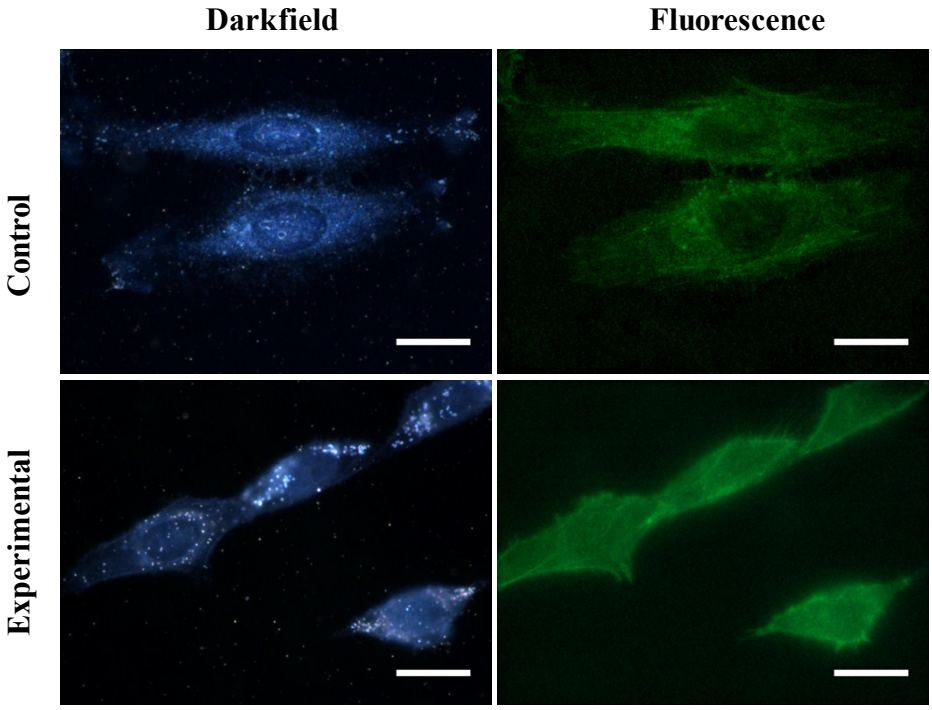

Figure S11. Examining the changes of cellular cytoskeletons for cells on hydrogel actuator without (Control, upper) or with (Experimental group, bottom) the NIR light irradiation at 24 mW/ mm^2. All scale bars equal 25 μm.

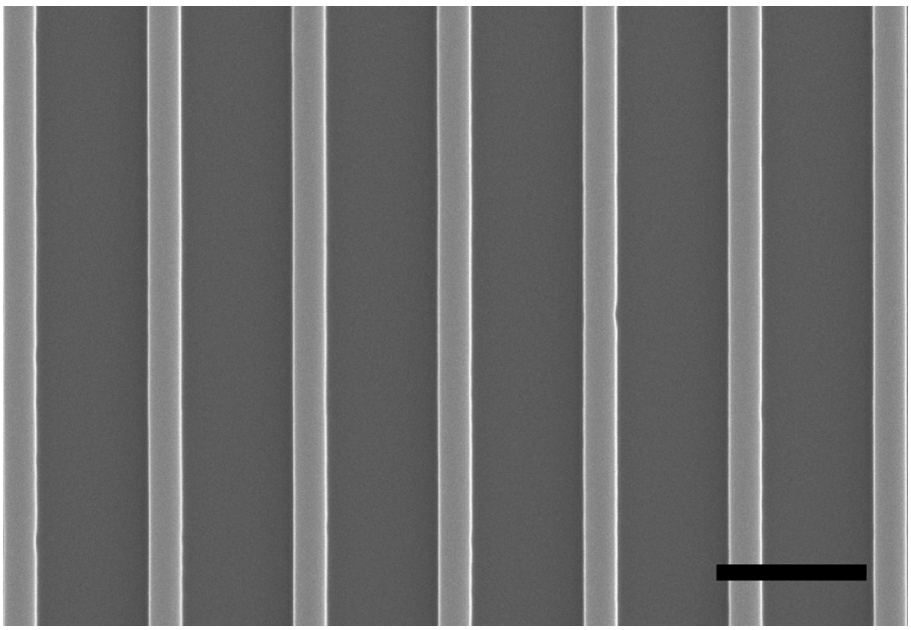

Figure S12. TEM characterization of the PDMS stamp used in the microcontact printing experiments.

The scale bar is 20 μm.