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Supporting information for

NIR-laser-triggered smart full-polymer nanogels for synergic photothermal-/chemo-therapy of tumor

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1. Experimental details

1.1 Materials.

N-Isopropylacrylamide (NIPAAm, 97% Sigma) was purified by re-crystallization from the mixture of hexane and benzene (1:4 v/v) and dried in vacuum. Chitosan (CS, low molecular weight, deacetylated chitin degree), pyrrole, *N,N'*-Methylenebis(acrylamide) (MBA) and iron(III) chloride hexahydrate were all purchased from Sigma-Aldrich Chemical Co., Ltd., USA. Sodium dodecyl sulfate (SDS) and potassium persulfate (KPS) were obtained from Sinopharm Chemical Reagent Co., Ltd., China. Doxorubicin hydrochloride (DOX) was supplied by Huafeng United Technology Co., Ltd., China. All other reagents were used as received without further purification. Ultrapure water was obtained from a Milli-Q system (Health Force Bio-meditech Holdings Ltd.).

1.2 Cell Experiments

Cell culture. The human hela cells were originally obtained from Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. Hela cells were grown in RMPI 1640 culture medium containing 10% fetal bovine serum, 100 units/mL

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penicillin, and 100 mg/mL streptomycin in a humidified incubator under 5% CO_2, at 37 °C.

Cytotoxicity. Cytotoxicity of PNA-CS-PPy-DOX nanogels were tested *in vitro* by the CCK-8 cytotoxicity assay (Dojindo) according to our previous study.[1] Hela cells were seeded into 96-well plates at a density of 1×10^4 cell/ well with 200 μL 1640 culture medium respectively at 37 °C in 5% CO_2 to allow the cell to attach. After 24 h incubation, the medium was replaced with 200 μL fresh 1640 culture medium containing PNA-CS-PPy or PNA-CS-PPy-DOX nanogels with various concentrations (0, 50, 100, 200, 400, 800 to 2400 μg/mL) for further 24 h, no PNA-CS-PPy-DOX as control. Then culture medium containing 10% of CCK-8 reagent was added to each well and incubated for 1 h. The spectrophotometric absorbance of the assay was measured using the microplate reader (iMark Microplate, Absorbance Reader, Bio-Rad Laboratories, Inc., USA) at 450 nm to determine the cell viabilities. Six parallel wells were done for each group. Cell viability was calculated as equation (1):

$$\mathbf{CV\%=100\times(Abs_{sample}-Abs_{blank})/(Abs_{control}-Abs_{blank})} \qquad (1)$$

Where Abs_{sample} is the absorbance at 450 nm for sample, and Abs_{blank} is the absorbance for blank without PNA-CS-PPy-DOX or PNA-CS-PPy and cells, as well as $Abs_{control}$ is the absorbance for control without PNA-CS-PPy-DOX or PNA-CS-PPy.

1.3 *In vitro*, photothermal and chemo-photothermal cancer therapy.

Hela cells suspension was dispensed in 200 μL centrifuge tube with a density of 1×10^5 cell/ tube. To directly demonstrate the therapeutic efficacy, the medium was respectively replaced by 200 μL 1640 culture medium containing samples of 70 μg/mL

DOX (without NIR laser treatment, as free DOX group), 10 mg/mL PNA-CS-PPy (subsequently for NIR laser treatment, as PNA-CS-PPy group), 10 mg/mL PNA-CS-PPy-DOX (LC_{DOX}=0.7%, subsequently for NIR laser treatment, as PNA-CS-PPy-DOX+NIR group), and the medium only with 1640 culture medium as control. Additionally, another PNA-CS-PPy-DOX were dispersed in 1640 culture mediums (10 mg/mL, LC_{DOX}=0.7%) in 200 μL centrifuge tube (without cells). Then these samples were irradiated by 915 nm laser (intensity 2.0 W/cm^2) to for 6 min. The supernatant solutions containing the released DOX were collected after centrifuged at 9000 rpm for 15 min, and then were used to replace the medium (as delivery DOX group). In subsequent NIR laser treatment, samples from PNA-CS-PPy+NIR groups, PNA-CS-PPy-DOX+NIR groups were irradiated by 915 nm laser (intensity 2.0 W/cm^2) to ensure temperature of 48 °C for 6 min. After that, cells from all four groups were seeded into 96-well plates at a density of 1×10^4 cell/ well with 200 μL 1640 culture medium at 37 °C in 5% CO_2 for 24 h. More than six parallel wells were done for each group to evaluate the cell viability by CCK-8. The remainder samples were seeded into 48-well plates at a density of 4×10^4 cell/ well in 1640 culture medium at 37 °C in 5% CO_2 for 24 h. Then, these cells were washed with PBS, and stained using a LIVE/DEAD kit (Invitrogen) for 30 min, according to the manufacturer's instructions and then imaged by a confocal fluorescence microscope (Leica TCS SP8, Leica Microsystems). Three replicates were done for each treatment group.

1.4 Animal Experiments

Animals and tumor model. Animal experiments were performed according to the Guidelines for institutional committee for animal use and care regulations. BALB/c nude

mice (6 weeks old, 15-20 g, male) were obtained from shanghai SLAC Laboratory Animal Center (Shanghai, China). Hela cells (5×10^6/mouse) were injected subcutaneously into the backside of each mouse to prepare tumor-bearing mice.

When the tumors achieved a surface diameter of 5-6 mm measuring with a digital caliper (about 10 days), mice were randomly distributed into five groups containing (n=6) (a) PBS, (b) PNA-CS-PPy-DOX, (c) DOX, (d) PNA-CS-PPy+NIR and (e) PNA-CS-PPy-DOX+NIR group, and then correspondingly intratumorally injected as following (a)100 μL PBS; (b) and (e) 100 μL PBS solution of PNA-CS-PPy-DOX (20 mg/mL, LC_{DOX}=0.7 %); (d) 100 μL PBS solution of PNA-CS-PPy (20 mg/mL); (c) 100 μL PBS solution of DOX (140 μg/mL) to each group, in order to ensure both PNA-CS-PPy-DOX and PNA-CS-PPy-DOX +NIR group have an equivalent DOX dosage to the free DOX group and an equivalent PNA-CS-PPy dosage to the PNA-CS-PPy group. After that, tumors from (d) PNA-CS-PPy+NIR and (e) PNA-CS-PPy-DOX+NIR groups were exposed to 915 nm laser at 2.0 W/cm^2 maintaining 47-48 °C for 10 min at 24 h post-injection. During this process, the temperature of the tumors-bearing mice was monitored and imaged simultaneously by the infrared thermal imaging camera. After 48h, mice were sacrificed and the tumors were collected for further histology analysis. All the experiments were carried out at room temperature of 25 °C.

Histology Analysis. Tumors, livers or muscles were harvested, and fixed in 10% neutral buffered formalin for 24 h, and then stained with hematoxylin and eosin (H&E) for further histology analysis.

Statistical Analysis. The data of the experiments were presented as mean ± standard

deviation (SD). Multiple comparisons among groups were determined using one-way ANOVA analysis followed by Tukey's post-test; *$P<0.05$ was represented a significant difference.

2. Figures

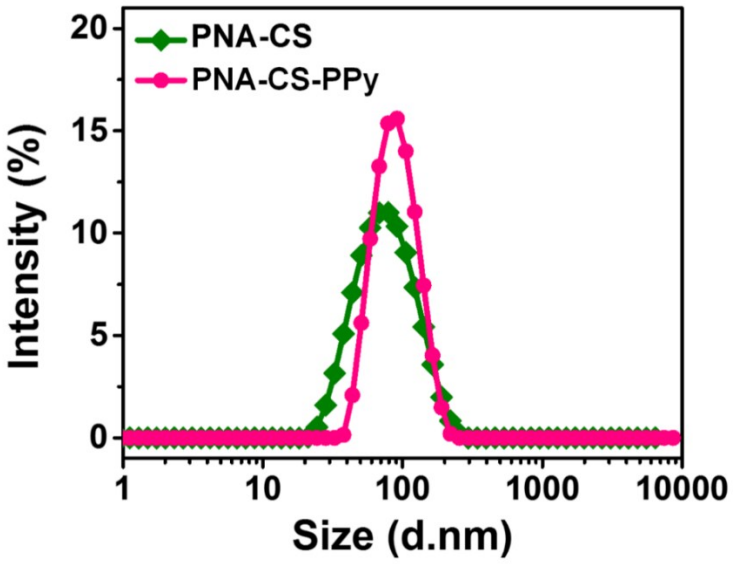

Figure S1. Size distribution of PNA-CS and PNA-CS-PPy nanogels.

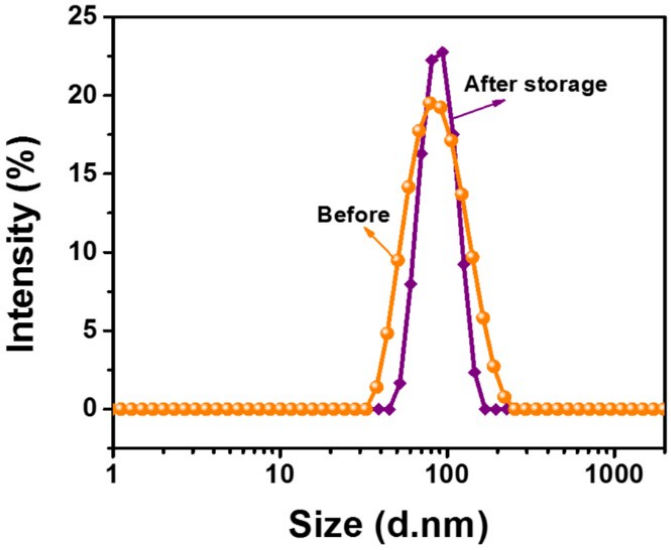

Figure S2. Size distribution of PNA-CS-PPy-DOX nanogels measured before and after stored for 4 weeks.

The diameter of PNA-CS-PPy-DOX nanogels were measured before and after stored for 4 weeks (**Figure S2**). Obviously, after 4 weeks, PNA-CS-PPy-DOX nanogels exhibit the average hydrodynamic diameter of about ~92±13 nm, which is close to the diameter of ~85±15 nm (before storage), verifying good long-term stability.

References

1 Z. Meng, F. Wei, R. Wang, M. Xia, Z. Chen, H. Wangand M. Zhu, *Adv. Mater.*, 2016, **28**, 245-253.