Supporting Information

Polydopamine-assisted decoration of Se nanoparticles on curcumin-incorporated nanofiber matrices for localized synergistic tumor-wound therapy

Meng Zhang,[a] Jiting Zhang, [a] Siyi Ran, [a] Wei Sun, [b] and Zhihong Zhu [,a]*

[a] Institute of Nano-Science and Nano-Technology, College of Physical Science and Technology, Central China Normal University, Wuhan, 430079, China.

[b] Key Laboratory of Laser Technology and Optoelectronic Functional Materials of Hainan Province, College of Chemistry and Chemical Engineering, Hainan Normal University, Haikou, 571158, China.

* To whom correspondence should be addressed.

Zhihong Zhu, E-mail: zhzhu@mail.ccnu.edu.cn

Experimental Section

Materials. PCL (Mw = 80000) was purchased from Shanghai yuanye Bio-Technology Co., Ltd. CS (Mw = 110000 - 150000), sodium selenite (Na_2SeO_3) and dopamine hydrochloride were purchased from Sigma-Aldrich Company (China). Acetic acid, ascorbic acid and CUR were all obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Tris[hydroxymethyl]-aminomethane (Tris-HCl) was purchased from Guangzhou Saiguo Biotechnology Co., Ltd. All reagents were used without further purification.

Preparation of CS-Stabilized SeNPs. Before the experiments, 0.8 mg/mL of CS solution was prepared by dissolving CS powder in 1% of acetic acid solution. The stock solution of Na_2SeO_3 (100 mM) and ascorbic acid (Vc) (100 mM) were freshly prepared. Briefly, 2.4 mL of Na_2SeO_3 (100 mM) was mixed with 9.6 mL of ascorbic acid (100 mM). After 15 min, 4.8 mL of CS (0.8 mg/mL) was added gradually into the mixture; then, it was reconstituted to a final volume of 80 mL with Milli-Q water and reacted overnight. Afterward, the solution was dialyzed using a dialysis bag (MW: 8000-14000) against Milli-Q water to remove unreacted materials. The prepared CS-stabilized Se nanoparticles were named as CS-SeNPs.

Fabrication of PCL/CUR Nanofiber Matrices via Electrospinning. The PCL solution with an optimized concentration (30 w/v%) was prepared by dissolving PCL in 5 mL glacial acetic acid. CUR (5 wt% relative to PCL) was blended with the PCL solution, stirred continuously for 2 h, and fed in a 5 mL syringe for subsequent electrospinning. The electrospinning parameters were as follows: electric field

strength: 8-10 kV; air gap distance: 16 cm; needle diameter of spinneret: 20 G; flow rate of solution: 0.15 mm/min. The electrospinning process was conducted at room temperature in air and performed using commercial electrospinning equipment (ET-2535H, Beijing Ucalery Technology Development Co., Ltd.). Finally, the as-spun fibres were air-dried for 48 h to remove the residual solvent. The obtained nanofiber mats were referred to as PCL/CUR. Unloaded PCL mats were also prepared under same electrospinning condition and called as PCL.

PDA Functionalization of PCL/CUR Matrices. Typically, the pre-prepared PCL/CUR fibres were placed in dopamine hydrochloride solution at a concentration of 2 mg/mL. The pH of solution was maintained to 8.5 via addition of Tris-HCl buffer (10 mM). Later, the above solution was filtered with a circulating water vacuum pump (SHZ-DIII) for 10 min to remove air from the PCL/CUR scaffolds. After being continuously stirred at 37 °C for 8 h in the dark, the membranes were collected and thoroughly rinsed with deionized water to remove unattached dopamine molecules. The PDA-coated PCL/CUR membranes were named PCL/CUR/PDA. For comparison, PDA-coated PCL fibres were also prepared through the exact same procedures as described above and named PCL/PDA.

Preparation of CS-SeNPs Decorated PCL/CUR/PDA Matrices. Briefly, 0.02 g of CS-SeNPs was ultrasonically dispersed in 100 mL deionized water to form uniform orange solution. Then, PCL/CUR/PDA fibres were immersed in above suspension and stirred for 6 h. When the solution turned transparent, the nanofiber matrices were collected and washed sufficiently through deionized water. The

obtained CS-SeNPs decorated PCL/CUR/PDA matrices were named as PCL/CUR/PDA@Se and lyophilized before use.

Surface Characterization. The as-prepared CS-SeNPs and various nanofiber matrices were characterized by SEM (JSM-6700F, Japan). The crystal structure and phases of the samples were characterized by XRD (X'Pert PRO MRD, PANalytical, Netherlands). The surface wettability of nanofiber matrices was measured using a Model 200 video-based optical system (Future Scientific Ltd. Co., Taiwan, China). FTIR analysis was performed using a Thermo Scientific Nicolet iN10 FTIR Microscope (Thermo Nicolet Corporation, Madison, WI) to identify the chemical structure of CS-SeNPs and nanofiber matrices.

***In Vitro* CUR Release.** PCL/CUR and PCL/CUR/PDA matrices of area 1 cm^2 were soaked in 10 mL of PBS of three different pH values (pH 7.4, 6.8 and 5.5) with 60% ethanol as an auxiliary solvent for CUR and incubated at 37 °C. At predetermined time, 1 mL of incubation solution was taken out and replaced with an equal volume of fresh PBS with different pH values. The CUR concentration in the collected medium was measured by ultraviolet-visible spectrophotometer (UV-vis, Lambda 750S, PerkinElmer, USA) at λ_{max} of 428 nm. At the end of the release experiments, the CUR retained in the respective mats was extracted using methanol as a solvent with extra sonication, and the CUR concentration was also assayed using UV-vis spectrophotometry. The amount of CUR was calculated by calibration curves constructed from known concentrations of CUR in PBS solution with different pH values.

In Vitro **Antioxidant Property Evaluation.** The antioxidant capacity of the matrices was determined via the scavenging DPPH free radical method. Various nanofibrous matrices with area of 1 cm^2 were dispersed in 2 mL of ethanol and then were ultrasonized for 20 min. A 0.2 mM of DPPH radical solution in ethanol was prepared, and then 2 mL of this solution was mixed with 2 mL of the above matrix precursor solution. Next, the mixture was stirred and incubated in the dark for 30 min. The remaining DPPH was scanned by a UV-vis spectrophotometer at 517 nm. The scavenging rate of DPPH was calculated by the formula:

$$DPPH\ scavenging\ \% = \frac{A_B - A_H}{A_B} \times 100\ \%$$

where A_B and A_H are the absorption of the blank (DPPH + ethanol) and the absorption of the various matrices (DPPH + ethanol + nanofibrous matrix) respectively.

Cell Viability and Cytotoxicity Assay. MG63 cells and NIH-3T3 cells were obtained from China Center for Type Culture Collection (Wuhan, China) and were cultured in DMEM medium containing 10 vol% fetal bovine serum (HyClone) and 1 vol% penicillin-streptomycin (Invitrogen) at 37 °C in a 5% CO_2 atmosphere incubator. MG63 and NIH-3T3 cells were seeded in 96-well plates and cultured at 37 °C and 5% CO_2 for 24 h. Subsequently, PCL, PCL/PDA, PCL/CUR, PCL/CUR/PDA and PCL/CUR/PDA@Se fibres were gently transferred into each well. After incubation for 1, 2 and 3 days, respectively, a standard MTT assay (MTT kit, Sigma, USA) was applied to measure the cell viability of each group. LDH activity was detected to assess the cytotoxicity. MG63 and NIH-3T3 cells were seeded in 96-well plates and cultured at 37 °C and 5% CO_2 for 24 h. Then the medium was replaced by fresh

serum-free medium containing PCL, PCL/PDA, PCL/CUR, PCL/CUR/PDA and PCL/CUR/PDA@Se fibers. After 1, 2 and 3 days of incubation, the medium were collected and centrifuged at 400 g for 5 min. Next, 120 μL of the medium supernatants were added to a fresh 96-well plates and incubated with 60 μL of LDH reagent for 30 min at 25 °C. The absorbance was measured using a microplate reader at 492 nm (DNM-9602).

Cell Morphology Observation. To observe the morphology and adhesion of cells on membranes, MG63 and NIH-3T3 cells were seeded on the surface of different nanofiber matrices. After incubation for 2 days, the cells were fixed with 3% glutaraldehyde for 1 h, followed by dehydrated through a graded ethanol series. Finally, the matrices were freeze-dried and observed under SEM. To evaluate the effect of composite membranes on the surrounding cells, the morphology of MG63 and NIH-3T3 cells cultured with different nanofiber matrices was further observed using a fluorescence microscope (NiKon-H550S, Japan). In brief, cells were seeded in 24-well plates containing glass slides and incubated overnight. Then, fresh culture media containing PCL, PCL/PDA, PCL/CUR, PCL/CUR/PDA and PCL/CUR/PDA@Se composite matrices were added and incubated for another 2 days. Afterwards, the cells were washed with PBS, and fixed with 3% glutaraldehyde for 15 min. After permeabilized with 0.5% Triton X-100 for 10 min, the cell cytoskeleton and nuclei were stained with Phalloidin-Alexa Fluor-555 and 4, 6-diamidino-2-phenylindole (DAPI) for 20 min, respectively, and observed by fluorescence microscopy.

Live/Dead Staining. MG63 and NIH-3T3 cells were seeded in 24-well plates containing glass slides and cultured overnight. Then, fresh culture media containing PCL, PCL/PDA, PCL/CUR, PCL/CUR/PDA and PCL/CUR/PDA@Se composite fibres were added and cultured for 2 days. Afterwards, cells were rinsed with PBS and stained with the mixed solution of Calcein-AM (2 mM in culture medium) and Ethidium homodimer-1 (4 mM in culture medium). After being cultured for 20 min at 37 °C, the samples were washed with serum-free medium and observed using a fluorescence microscope.

Mitochondrial Membrane Potential (ΔΨm) Assessment. A mitochondrial potential-sensitive probe, JC-1, was used to evaluate the changes of ΔΨm. Briefly, MG63 cells were seeded in 24-well plates containing glass slides and cultured overnight. PCL, PCL/PDA, PCL/CUR, PCL/CUR/PDA and PCL/CUR/PDA@Se composite fibres were incubated with the cells for 2 days at 37 °C. After that, cells were washed twice with PBS and then stained with JC-1 according to the manufacturer's instructions. The fluorescent images of staining cells were captured using a fluorescence microscope.

ROS Generation Assay. MG63 cells were seeded in 24-well plates containing glass slides and cultured overnight. PCL, PCL/PDA, PCL/CUR, PCL/CUR/PDA and PCL/CUR/PDA@Se composite fibres were incubated with the cells for 2 days at 37 °C. Next, the cells were washed with pre-cooled PBS, and exposed to DCFH-DA (10 μM in serum-free medium), followed by additional incubation for 30 min at 37 °C.

Finally, cells were washed repeatedly with serum-free medium, and the intracellular ROS levels were analyzed using a fluorescence microscopy.

***In Vivo* Tumor Therapy and Wound Healing.** All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Central China Normal University and approved by the Animal Ethics Committee of the Hospital of Central China Normal University affiliated with Central China Normal University. The nude mice (BALB/c, 5 weeks old) purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. (China; animal quality certificate NO.1100111911049644). UMR-106 cells (1×10^6 cells suspended in 100 μL of serum-free RPMI-1640 medium) were first subcutaneously injected into the back of each mouse were used to establish the tumor models. When tumor volume reached approximately 80 mm^3, the mice were divided into three groups, randomly (n = 4): Control (without any treatment), PCL, and PCL/CUR/PDA@Se. Prior to the *in vivo* implantation, a full thickness wound (diameter: 5 mm) was created at above the tumor site. Subsequently, the exposed tumor in the experimental groups was covered by the PCL and PCL/CUR/PDA@Se nanofiber matrices with the dimension of 6 mm × 6 mm. The detail steps could be seen in Fig. S1. The tumor size was measured and the wound was photographed every few days. The tumor volume was calculated as the formula:

$$tumor\ volume = \frac{(tumor\ length) \times (tumor\ with)^2}{2}$$

After 14 days of treatment, the mice were sacrificed and all tumors were harvested for weighing, photographing. The tumor tissues were stained with H&E, TUNEL, and

Ki67 for histological analysis. In order to investigate the wound healing efficacy of composite membranes in tumor-bearing mice, the skin tissues at the wound beds and surrounding healthy skins were collected and evaluated using H&E staining.

Statistical Analysis. Each experiment was repeated three times. For samples that were used for each experiment, an n = 5 or 6 was used. All data used were analyzed using the statistical software Origin 8.0 and expressed as the means ± standard deviations (SD). The significance of differences between two groups was performed using one-way ANOVA and then Student's *t* test used to evaluate. The p-value < 0.05 was considered to be the significant difference.

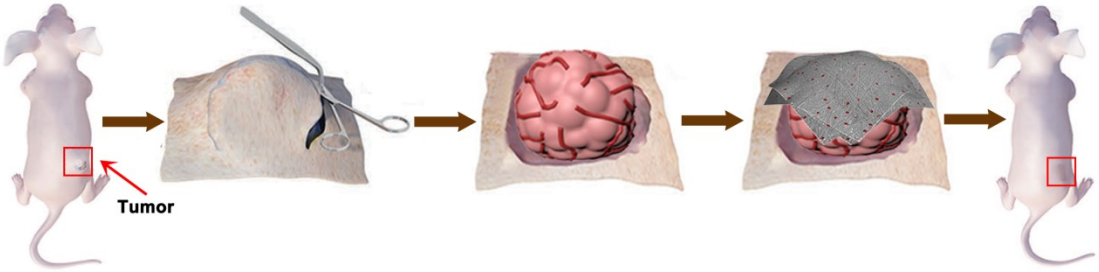

Fig. S1 Schematic representation of the surgical procedure.

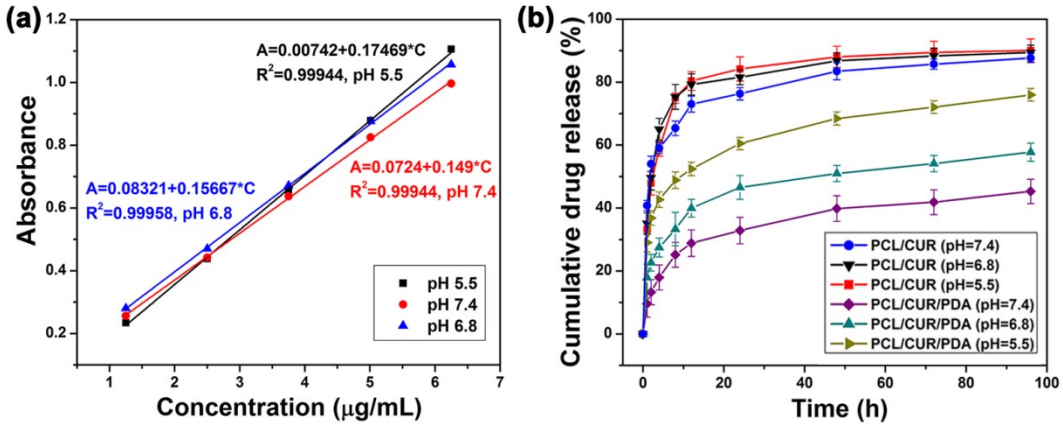

Fig. S2 (a) UV-vis calibration curve of CUR under different pHs. (b) Cumulative CUR release of PCL/CUR and PCL/CUR/PDA matrices under different pH values within 12 h.

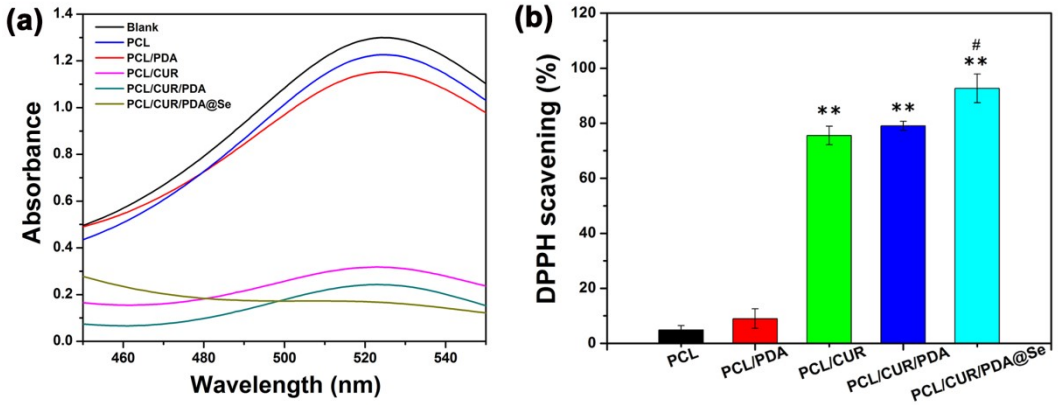

Fig. S3 (a) UV-vis spectra of DPPH and DPPH after scavenged by nanofiber matrices for 30 min. (b) Antioxidant property demonstrated by DPPH scavenging ratio of different nanofiber matrices.

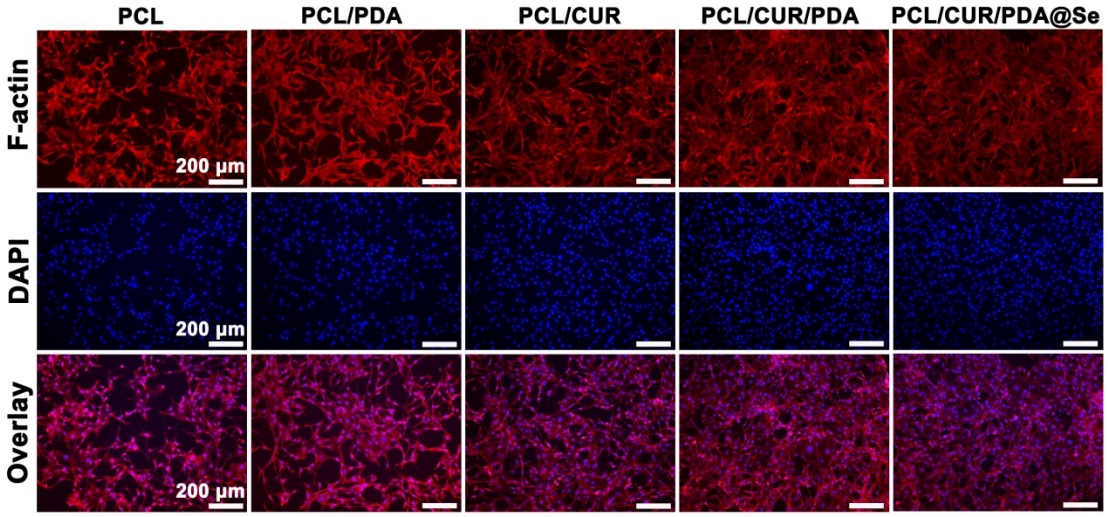

Fig. S4 F-actin morphology of NIH-3T3 cells cultured with different nanofiber matrices.

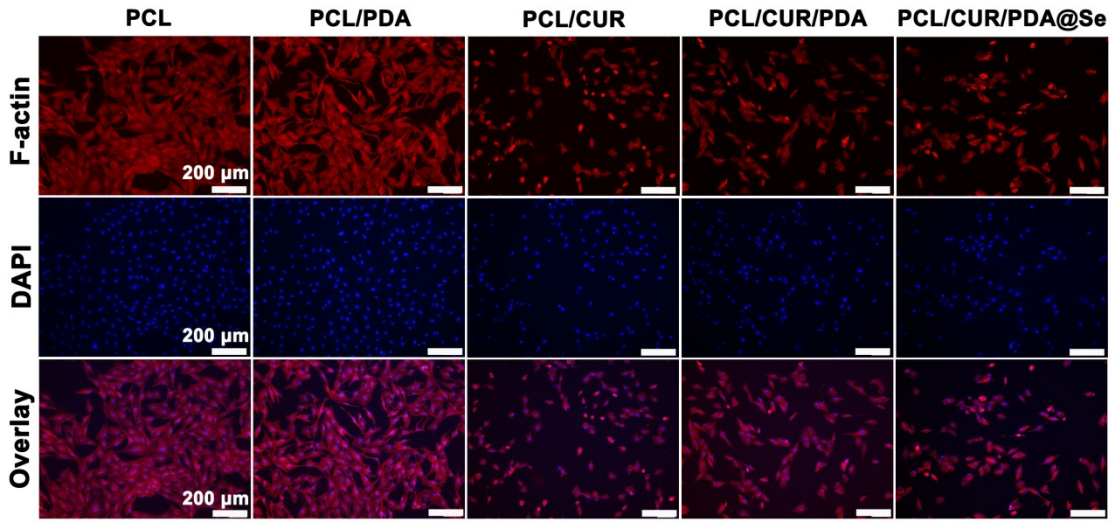

Fig. S5 F-actin morphology of MG63 cells cultured with different nanofiber matrices

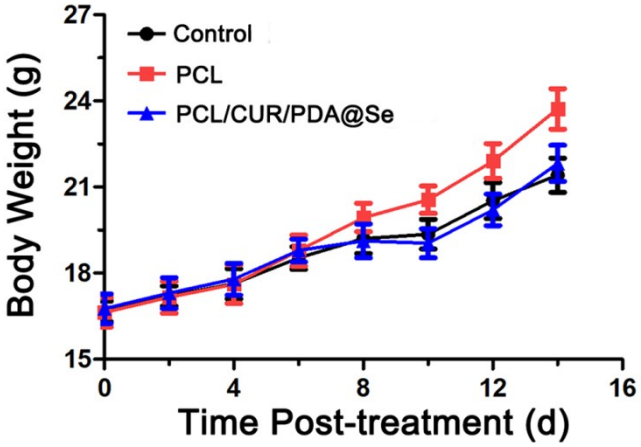

Fig. S6 Change in body weight of tumor-bearing mice.