SUPPORTING INFORMATION

Reduced Graphene Oxide Embedded Polymeric Nanofiber Mats: An 'On-Demand' Photothermally-Triggered Antibiotic Release Platform

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Characterization.

Scanning Electron Microscopy (SEM) images were obtained using an electron microscope ULTRA 55 (Zeiss, France) equipped with a thermal field emission emitter and three different detectors (EsB detector with filter grid, high efficiency In-lens SE detector and Everhart-Thornley Secondary Electron Detector). A Horiba Jobin Yvon LabRam high resolution micro-Raman system combined with a 473-nm (1 mW) laser diode as excitation source was used for Micro-Raman spectroscopy measurements. Visible light was focused by a 100× objective. Using a backscattering configuration, the scattered light was collected by the same objective, dispersed by a 1800 mm focal length monochromator and detected using CCD camera. A Thermo Scientific Nicolet 380 FT-IR spectrophotometer was used for obtaining Fourier transform infrared spectrum. Kratos Analytical Axis Ultra X-ray photoelectron spectrometer (XPS/ESCA) with an Al Kα-mono-chromatic source of 1486.71 eV was used for recording XPS spectra.

Measurement of the photothermal effect. All irradiations were performed in standard 96-well plates. For photothermal experiments, a 980 nm-continuous wave laser (Gbox

model, Fournier Medical Solution) was used at laser power densities between 0.5 and 2 W cm^{-2}. This laser was injected into a 400 μm-core fiber and placed at 6 cm from the bottom of the wells. The output was non-collimated and resulting beam divergence allows uniform illumination of 4 wells. The temperature changes on the surface of the nanofiber mats were captured by an infrared camera (Thermovision A40) and processed with a ThermaCam Researcher Pro 2.9 software.

High-performance Liquid Chromatography (HPLC) for Quantification of Ampicillin and Cefepime. The concentration of ampicillin and cefepime loaded PAA@rGO was determined by an HPLC system (Shimadzu, Tokyo, Japan) equipped with a 5 μm C_4 QS Uptisphere® 300 Å, 250 mm × 4.6 mm column (Interchim, Montluçon, France) heated to 40 °C. The mobile phase consisted of a mixture of eluent A (trifluoroacetic acid 0.1% in water) and eluent B (trifluoroacetic acid 0.1 % in acetonitrile) at a flow rate of 1 mL/min. The samples were injected at a volume of 40 μL and the detection at a wavelength of 227 nm.

Bacterial Growth Conditions. The Gram-negative *E. coli* K12 were grown at 37 °C with shaking in Luria Bertani (LB) broth overnight. Upon 100-fold dilution, incubation was prolonged until the OD_{600} had reached 0.3-0.5. *S. epidermidis,* a clinical strain obtained from the Central Hospital of Roubaix (France) and *S. aureus* (ATCC® 25923) were grown in brain heart infusion (BHI) medium at 37°C overnight without agitation.

Bacteria Cell Viability. Following release of ampicillin and cefepime, bacteria survival was measured by determining the titer of viable bacteria ability to grow. A 10-fold dilution series of the bacterial solutions in phosphate buffer saline were spotted in 10 µL aliquots on LB-agar medium. Visual counting of the number of colonies upon overnight incubation at 37 ºC allowed reading out the initial and final concentrations of the bacteria strains in cfu mL^{-1}.

Wound Healing and Histology. Studies involving animals, including housing and care, method of euthanasia, and experimental protocols were conducted in accordance with the local animal ethical committee in the animal house (Permission 20170918/8) of Danylo Halytsky Lviv National Medical University, under the supervision of Prof. R. Bilyy.

Six to 8-week-old male Balb/c mice were used for this investigation. The mice were housed in cages covered with air-filters in a temperature-controlled room with a 12 h light and 12 h dark schedule and kept on a standard diet with drinking water available *ad libitum*. All animal experiments were performed in accordance with institutional ethical guidelines. For the bacterial surface infection, mouse fur on the back was removed using MedaSept medical depilation cream. The superficial damaged skin infection model was performed as described us previously[49] with some modifications. After removing the fur, a plaster was applied to the mouse skin (1 cm^2) for 15 times to remove the superficial epidermis. The skin became visibly damaged and characterized by reddening and glistening but no regular bleeding. Then 10 µL of *S. aureus* bacterial cells (4×10^7 cfu mL^{-1}) were applied to the skin was allowed to dry for 10 min. Superficial skin infection established after 24 h and PAA or cefepime loaded PAA@rGO nanofiber mats were applied to the skin of anesthetized mice. Untreated mice were used as control. The mat was illuminated for 10 min with a Near-Infrared LED array (6×6 mm^2 in size, 8 W, 2A, 940 nm), previously shown to be a safe condition for animals.[49] The temperature was monitored by an infrared camera (Thermovision A40). Mice were photographed with a

Nikon camera, equipped with Micro-Nikkor objective, from a fixed position and with reference ruler present in each picture. Photos were processed with ImageJ software to detect erythema, and its area before and 24 h after treatment with patch was evaluated. After 24 h the mice were sacrificed, the skin was removed, photographed, and fixed with 4% neutral buffered formalin solution and embedded in paraffin according to the standard laboratory protocol. The sliced tissues were stained by hematoxylin and eosin (H&E) (Sigma-Aldrich) or Gram stain (Remel, Lenexa, KS) using routine laboratory procedures. Gram staining was performed by applying crystal violet to the tissue sections for 5 min at room temperature, and slides were rinsed to remove excess crystal violet. Gram iodine mordant was applied for 2 min to the tissue sections and washed in tap water. To remove any non-specific crystal violet staining, a Gram decolorizer solvent was applied to the slides for 30 s then quickly rinsed under running tap water until the water ran clear. The sections were then stained with Gram Safranin for 1 min and 40 s, followed by dehydration through a series of alcohol (95–100 %) to xylene and then coverslipped.

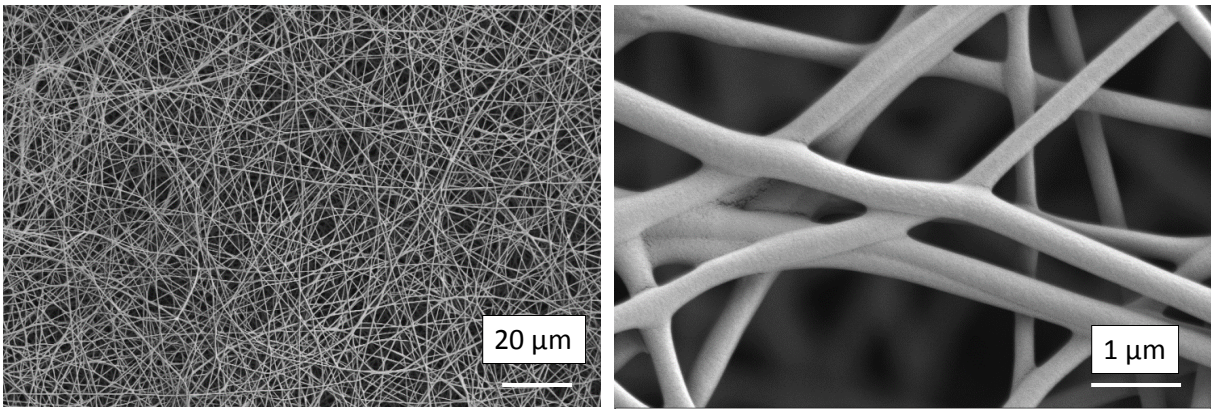

Figure S1. SEM images of fabricated PAA nanofiber mats at different scales.

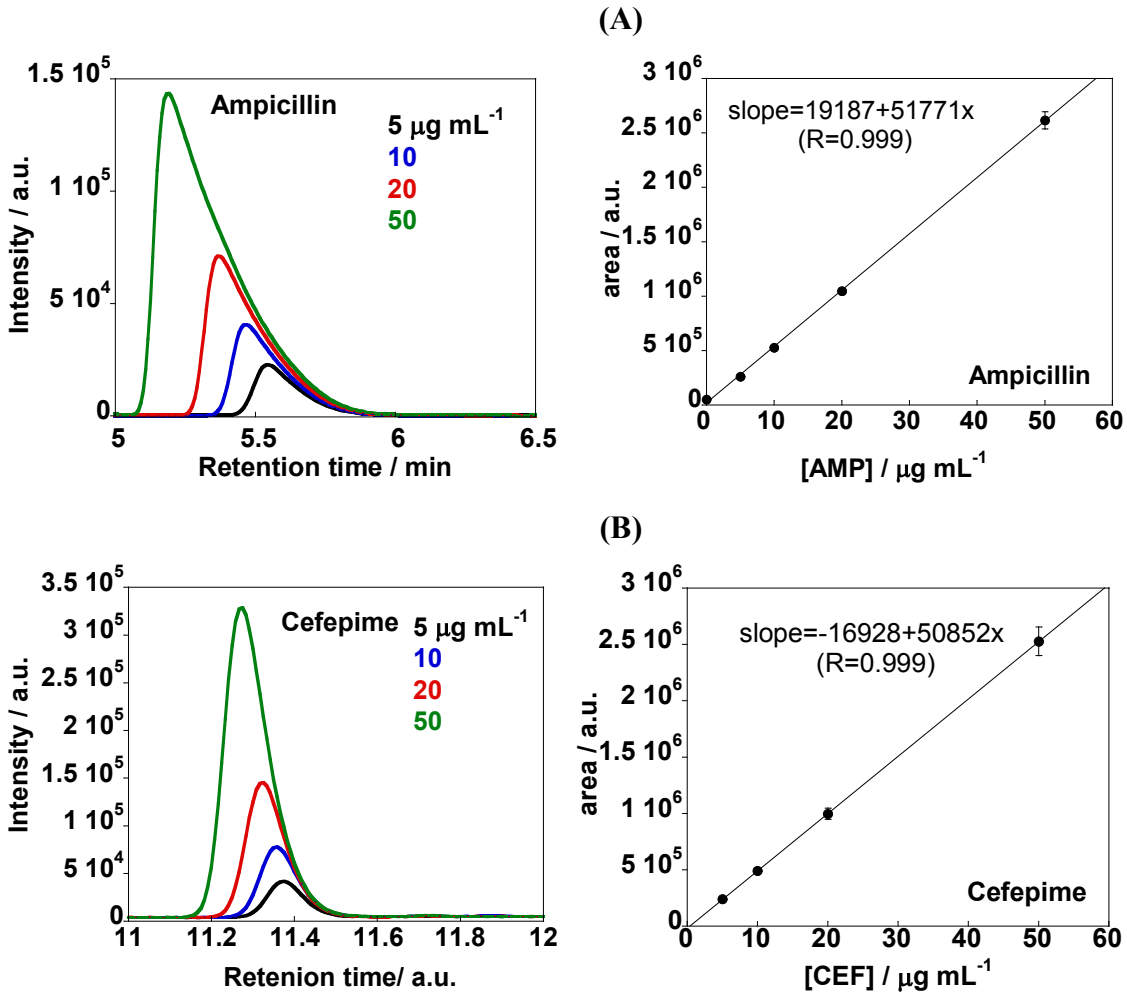

Figure S2: (A) HPLC plot of AMP (5, 10, 20 and 50 µg mL^{-1}) in PBS (0.1 M, pH 7.4) together with AMP calibration curve; (B) HPLC plot of CFP (5, 10, 20 and 50 µg mL^{-1}) in PBS (0.1 M, pH 7.4) together with CFP calibration curve.

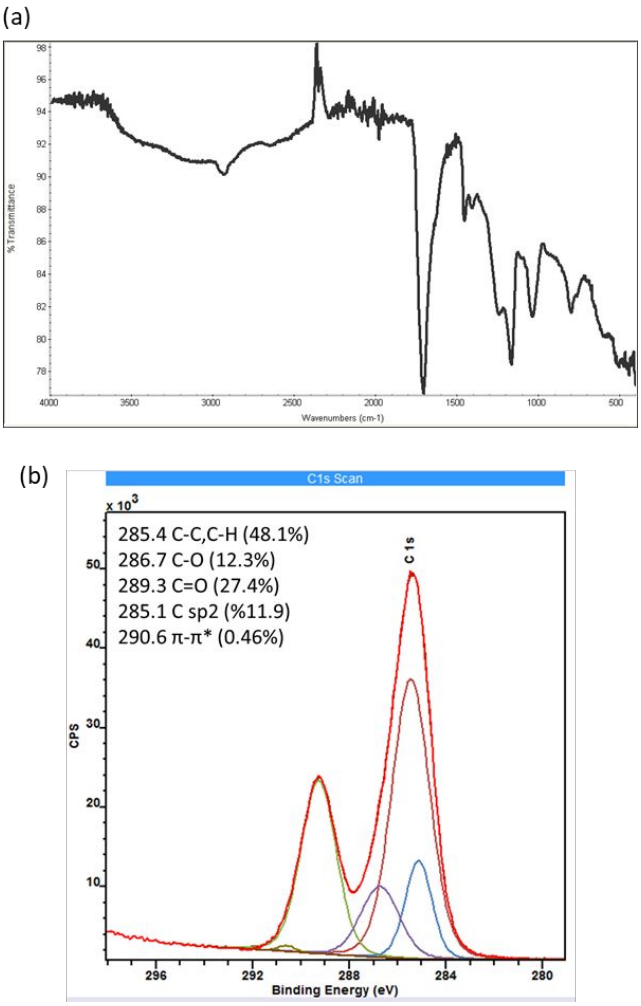

Figure S3. FTIR and XPS (C1s Scan) spectra of PAA@rGO nanofibers.

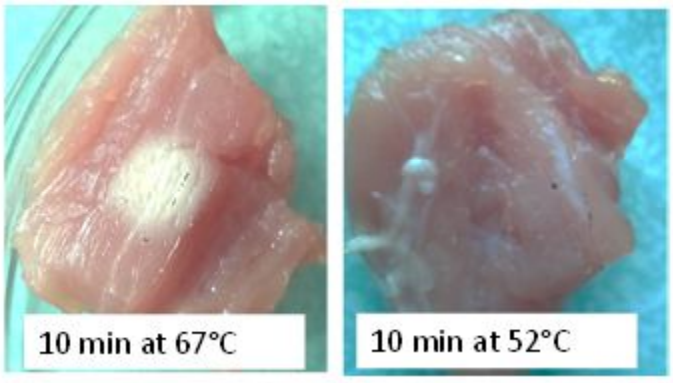

Figure S4. Controlled heating of meat with PAA@rGO with NIR LED: Heating up to 67°C for 10 min causes meat to burn, but heating skin to 52°C shows no visible tissue damage.