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

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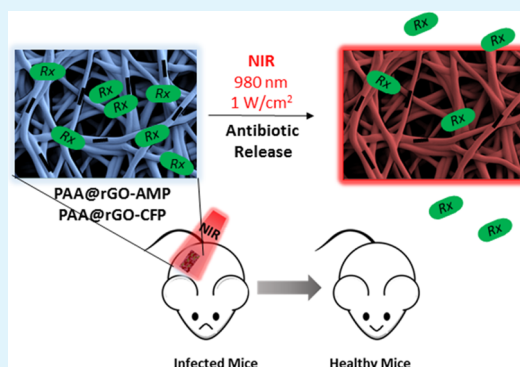
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Supporting Information

ABSTRACT: The steady increase in antimicrobial resistance in different pathogens requires the development of alternative treatment strategies next to the oral delivery of antibiotics. A photothermally activated platform based on reduced graphene oxide (rGO)-embedded polymeric nanofiber mats for on-demand release of antibiotics upon irradiation in the near-infrared is fabricated. Cross-linked hydrophilic nanofibers, obtained by electrospinning a mixture of poly(acrylic acid) (PAA) and rGO, show excellent stability in aqueous media. Importantly, these PAA@rGO nanofiber mats exhibit controlled photothermal heating upon irradiation at 980 nm. Nanofiber mats are efficiently loaded with antibiotics through simple immersion into corresponding antibiotics solutions. Whereas passive diffusion based release at room temperature is extremely low, photothermal activation results in increased release within few minutes, with release rates tunable through power density of the applied irradiation. The large difference over passive and active release, as well as the controlled turn-on of release allows regulation of the dosage of the antibiotics, as evidenced by the inhibition of planktonic bacteria growth. Treatment of superficial skin infections with the antibiotic-loaded nanofiber mats show efficient wound healing of the infected site. Facile fabrication and implementation of these photothermally active nanofiber mats makes this novel platform adaptable for on-demand delivery of various therapeutic agents.

KEYWORDS: electrospinning, nanofibers, reduced graphene oxide, antibiotic release, photothermal effect



INTRODUCTION

One of the challenges frequently encountered in traditional methods of drug delivery relates to poor control over proper dosage of therapeutic agent.^{1–3} Maintaining the drug concentration within a particular therapeutic window and below toxic concentrations is challenging, and lack of efficient treatment can lead to severe side effects, along with the development of drug resistance in cells and pathogens. These problems have fostered the development of different approaches that enable the controlled release of a precisely defined amount of drug, but allow in addition the release of the therapeutic dose on demand, upon the application of an external trigger rather than by passively driven diffusion

processes. In the last decade, several delivery systems have been fabricated to deliver drug locally by controlling release kinetics through utilization of various types of stimuli, either external or intrinsic to the disease site.^{4,5} The capability to tune the physicochemical properties of polymeric nanostructures as well as other advantages such as their stability in vitro and in vivo conditions and good biocompatibility has laid the foundation for the emergence of a large number of polymeric nanosystems for controlled drug release.^{6–9}

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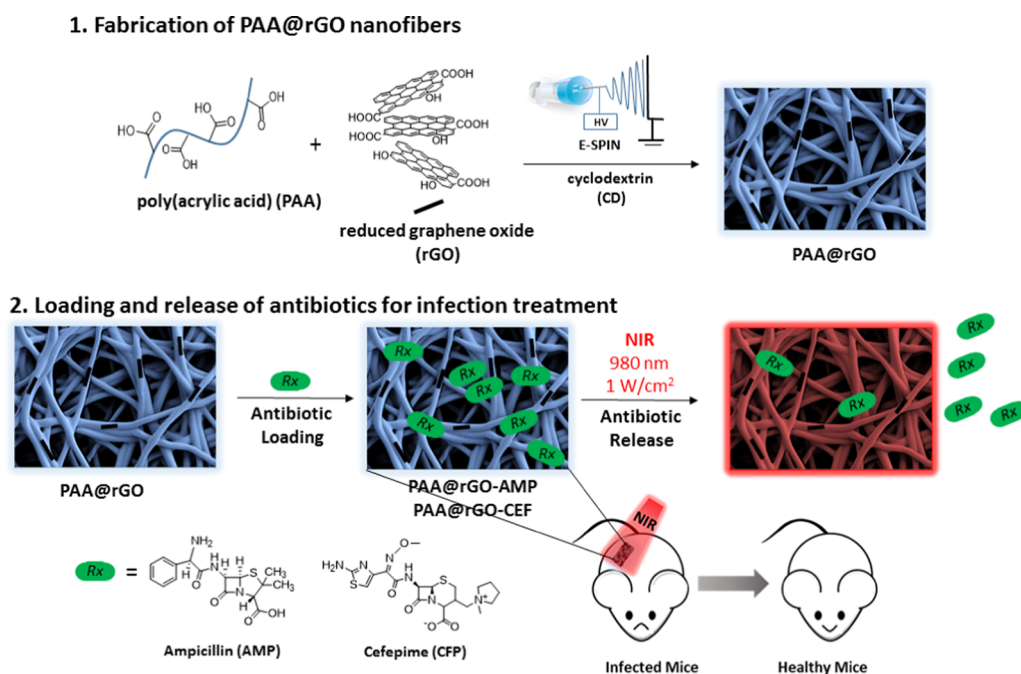


Figure 1. Illustration of the fabrication of antibiotic-loaded PAA@rGO nanofibers: (1) electrospinning of rGO-loaded PAA nanofiber mats followed by (2) loading with different antibiotics and photothermal-triggered antibiotic release.

Over the past decades, there is considerable evidence of the benefits polymeric materials as antibiotic delivery platform.^{10–12} Polymeric materials have shown to solve problems related to the solubility of hydrophobic antibiotics, as well as provide a protective coating against environmental degradation and deactivation through the body's clearing mechanisms. The structures proposed range from discrete soluble polymers conjugated with antibiotics¹³ to materials where polymers are chemically or physically assembled in the form of hydrogels,^{14–17} micelles,¹⁸ nanogels,¹⁹ nanoparticles,²⁰ and nanofibers.²¹ Polymeric nanoparticles are well suited for addressing bacterial infections inside the body as the nanometric size of antibiotic-loaded nanoparticles enables efficient penetration through the mucus layer around bacteria biofilms, thus increasing the local concentration of antibiotic in the biofilm.¹ On the other hand, antibiotic-loaded bandages, foams, and dressings are preferential for external applications such for the management of chronic wounds.^{22,23} In this regard, antibiotic-loaded nonwoven fibrous materials obtained using polymeric nanofibers could be ideal candidates for wound infection treatment, as they can be directly fabricated in the form of ready-to-apply materials. One of the most commonly encountered challenge in wound healing is infection. Next to iodine- or silver-ion-containing dressing, antibiotic ointments are regularly used to prevent wound infection.²⁴ In the case of antibiotic ointments, discomfort caused to the patient, along with the chances of developing resistance to antibiotics and contact dermatitis, has put their efficiency into question.²⁵

One attractive alternative as a wound-healing material and a protective scaffold after surgical procedures favoring site-specific therapy by increasing the local antibiotic concentration might be the use of antibacterial-loaded nanofiber mats. These polymeric scaffolds are engineered via a polymer-processing technique called electrospinning, which allows usage of a variety of polymers with different chemical composition and morphology.^{26–28} This enables to select the most appropriate material for the intended application and enables tailoring of

drug release profiles.²⁹ Hadjiargyrou and co-workers demonstrated the incorporation of hydrophilic antibiotics (mefoxin and cefoxitin) and their release in a sustained manner from poly(lactide-co-glycolide) nanofiber-based scaffolds obtained using electrospinning, without any compromise in their bioactivity and structure.³⁰ A passive diffusion release of about 70% of loaded cefoxitin was observed in the first hour, and the rest of the loaded antibiotic was released over 1 week. Although the antibiotic that was released from these nanofibers was effective in inhibiting *Staphylococcus aureus* growth (>90%), burst release is an undesirable feature. To achieve an antibiotic release at the constant rate over a prolonged time period, stimuli-responsive nanofibers have been designed, mainly using pH-based triggers.^{31,32} For example, ciprofloxacin-loaded pH-responsive electrospun nanofibers fabricated from a copolymer of vinyl benzoic acid and vinylbenzyltrimethylammonium chloride, as reported by Uyar and co-workers, undergo enhanced drug release at pH 5.8. Although external modulation of pH is nontrivial, modulation of temperature is unique, as it can be considered to be either internal stimuli as some infections are naturally at elevated temperature, or to be external through the application or generation of heat in the nanostructure. In the case of polymers, a temperature change can result in rapid swelling of its structures, leading to high volume alterations and thus inducing enhanced drug release.⁷ Some photothermal active nanofibers formed through the incorporation of gold nanostructures,^{33–35} carbon nanotubes,^{36,37} carbon nanoparticles,³⁸ or more recently Cu₂S³⁹ have been described. We have shown lately the interest in reduced graphene-oxide (rGO)-loaded hydrogels for photothermal-initiated insulin release.⁴⁰

Interestingly, although reports of graphene oxide (GO) and rGO-containing nanofibers have emerged in recent years,^{41–44} only one report used the photothermal properties of the resulting nanocomposite,⁴⁵ and showed photothermal eradication of captured cancer cells. The photothermally triggered

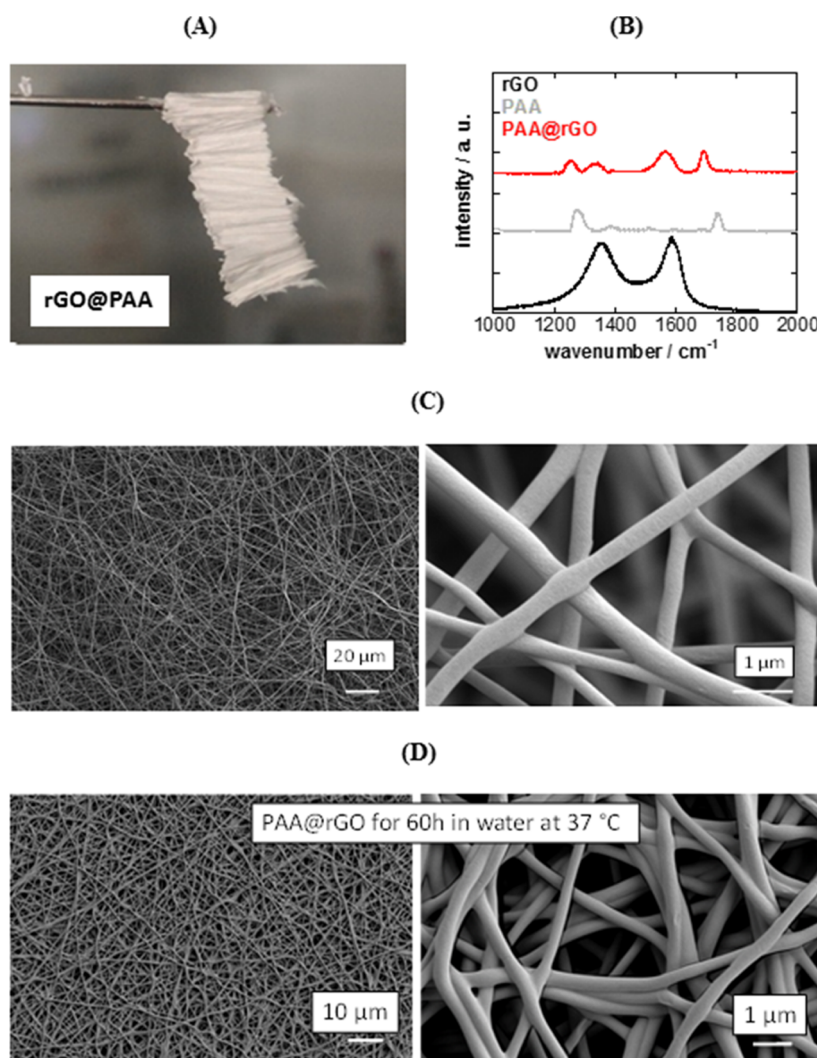


Figure 2. Characterization rGO-loaded PAA nanofiber mats formed by electrospinning: (A) photograph of electrospun PAA@rGO; (B) Raman spectra of rGO (black), PAA (gray), and PAA@rGO (red); (C) scanning electron microscopy (SEM) images of fabricated PAA@rGO nanofiber mats at different scales; and (D) stability of β -cyclodextrin cross-linked PAA@rGO nanofibers in water at 37 °C for 60 h.

131 release of antibiotics from rGO-containing nanofibers and its
 132 use for the treatment of infections has not been explored until
 133 now. Herein, we disclose rGO-containing poly(acrylic acid)
 134 (PAA) based nanofiber mats (PAA@rGO) for the loading of
 135 different antibiotics due to their swelling and noncovalent
 136 interaction with rGO, as well as demonstrate controlled and
 137 on-demand release promoted by near-infrared photothermal
 138 heating (Figure 1). To allow facile diffusion of antibiotics
 139 under aqueous environment into nanostructures, we opted for
 140 the formation of rGO-loaded hydrophilic nanofibers by
 141 electrospinning PAA in the presence of cyclodextrin as a
 142 cross-linker. These nanofiber mats showed controlled photo-
 143 thermal heating ability, with the temperature reaching 67 ± 2
 144 °C using a light source of 980 nm and 1 W cm^{-1} power
 145 density. The PAA@rGO nanomats showed high loading
 146 capability for ampicillin (AMP) and cefepime (CFP) with
 147 minimal passive antibiotic release. Ampicillin, part of the
 148 aminopenicillin family, was chosen for its favorable structure
 149 (aromatic ring together with the presence of amine and acid
 150 functional groups), enabling ampicillin to interact with rGO
 151 through electrostatic and π -stacking interactions (Figure 1).
 152 The same is true for cefepime (CFP), an antibiotic belonging

to the class of cephalosporin commonly applied for the
 treatment of bacterial conditions such as pneumonia, kidney,
 urinal, as well as skin infections. This antibiotic works by
 simply stopping the growth of bacteria.^{46,47} Enhanced drug
 release was achieved under irradiation of the antibiotic-loaded
 PAA@rGO nanofiber mats with near-infrared light at 980 nm,
 where the antibiotic release rate can be tuned upon using
 different power densities. In vitro antibacterial assay demon-
 strates clearly that the released antibiotics did not lose their
 biological activity to inhibit growth of pathogens. The
 nanofiber platform could be reloaded with antibiotic and
 release was achieved in a controlled fashion, thus demon-
 strating recyclability of the platform. Furthermore, through in vivo
 studies on *S. aureus* infected Balb/c mice, the antibiotic-loaded
 PAA@rGO nanofiber mats demonstrated excellent wound-
 healing capability, making them viable candidates for clinical
 applications.

EXPERIMENTAL SECTION

Materials. Poly(acrylic acid) (PAA, $M_n = 450\,000 \text{ g mol}^{-1}$), 171
 hydrazine, β -cyclodextrin, phosphate-buffered saline (PBS, pH 7.4) 172
 and cefepime were purchased from Sigma-Aldrich. Ampicillin (AMP) 173

174 was obtained from Fisher BioReagents (BP1760-25). Graphene oxide
175 (GO) in powder form was purchased from Graphenea (Spain).

176 **Characterizations and Photothermal Setup.** Details of
177 characterization techniques and instrumental details for photothermal
178 setup employed in this study can be found in the [Supplementary](#)
179 [Information](#).

180 **Fabrication of rGO-Loaded PAA Nanofiber (PAA@rGO).** A
181 GO precursor was chemically reduced to reduced graphene oxide
182 (rGO) using hydrazine according to a procedure previously reported
183 by us.^{40,48} Briefly, GO aqueous suspension (5 mL, 0.5 mg mL⁻¹) was
184 treated with hydrazine (0.50 mL, 32.1 mM) at 100 °C for 24 h. After
185 this time, the reduced GO gradually precipitates. Filtration through a
186 poly(vinylidene difluoride) membrane (0.45 μm pore size) is used to
187 collect the product. Obtained residue is washed with copious amounts
188 of water (5 × 20 mL) and methanol (5 × 20 mL). Finally, the product
189 is dried in an oven for 18 h at 100 °C. Thereafter, to rGO aqueous
190 suspension (1.2 mL, 2.5 mg mL⁻¹) was added PAA (100 mg) and β-
191 cyclodextrin (20 mg) and the resulting mixture was stirred for 24 h at
192 room temperature. This corresponds to rGO (2.4 wt %), PAA (81.4
193 wt %), and β-cyclodextrin (16.2 wt %). Clear polymer solution of
194 PAA, β-cyclodextrin, and rGO was electrospun using a 1 mL syringe
195 fitted with a 14-gauge blunt needle at the rate of 0.005 mL min⁻¹ with
196 15 kW. The distance was kept at 15 cm during the electrospinning
197 process. Resulting electrospun fibers were cross-linked under vacuum
198 at 140 °C for 30 min.

199 **Loading of Antibiotics onto Nanofibers.** Antibiotics loading
200 into the nanofibers was achieved by immersing the nanofiber mat (4
201 mg) into the aqueous solution of ampicillin (80–400 μg mL⁻¹) or
202 cefepime (80–400 μg mL⁻¹) and shaking (150 rpm) at 4 °C for 72 h.
203 The concentration of ampicillin and cefepime loaded into the
204 nanofibers was determined from the absorption intensity of the AMP
205 and cefepime remaining in the solution using high-performance liquid
206 chromatogram (HPLC). Ampicillin and cefepime calibration curves
207 were obtained using a series of antibiotic-containing solutions (1–100
208 μg mL⁻¹) ([Figure S2](#)).

209 **Photothermal-Triggered Antibiotics Release.** Antibiotic
210 release was assessed in phosphate-buffered saline (PBS) (1 mL, pH
211 7.4) through direct irradiation of the nanofibers using a continuous
212 wave laser at 980 nm at varying power densities (1–2 W cm⁻²) for
213 several minutes. The amount of antibiotics released was determined
214 from HPLC results of the solution collected after irradiation using
215 previously established calibrations curves ([Figure S2](#)). Control
216 experiments were carried out in an environmentally controlled
217 cabin at 37 °C, and the samples were gently shaken during incubation.

218 **Wound Healing and Histology.** Full details of the study can be
219 found in the [Supplementary Information](#). Briefly, 6–8-week-old male
220 Balb/c mice were used for this study. The superficial skin damaged
221 infection model was performed as described by us previously⁴⁹ with
222 some modifications. After removing the fur, a plaster was applied to
223 the mouse skin (1 cm²) several times to remove the superficial
224 epidermis. The skin damaged was visible and characterized by
225 reddening and glistening but no regular bleeding. Then, 10 μL of *S.*
226 *aureus* bacterial cells (4 × 10⁷ cfu mL⁻¹) were applied to the skin and
227 allowed to dry for 10 min. Superficial skin infection established after
228 24 h and PAA- or cefepime-loaded PAA@rGO nanofiber mats were
229 applied to the skin of the anesthetized mice. Untreated mice were
230 used as control. The mat was irradiated for 10 min with a near-
231 infrared light-emitting diode (LED) array (6 × 6 mm² in size, 8 W,
232 2A, 940 nm), previously shown to be a safe condition for animals.⁴⁹
233 The temperature was monitored by an infrared camera (Thermovi-
234 sion A40). Mice were photographed and images were processed with
235 ImageJ software to detect erythema, and its area before and after a 24
236 h treatment was evaluated. After 24 h, the mice were sacrificed and
237 the skin was removed and subjected to histological analysis.

238 ■ RESULTS AND DISCUSSIONS

239 **Fabrication and Characterization of rGO-Containing**
240 **Nanofibers.** First, rGO-containing PAA nanofibers were
241 obtained through electrospinning of an aqueous dispersion of

rGO (2.4 wt %), PAA (81.4 wt %), and β-cyclodextrin (16.2 wt 242
%) as a cross-linker ([Figure 1](#)). Upon electrospinning, a free 243
standing mat of electrospun fibers is obtained ([Figure 2A](#)). 244
Raman spectra of PAA and PAA@rGO were recorded to 245
confirm the incorporation of rGO into the nanofibers ([Figure](#)
246 [2B](#)). Raman analysis revealed the presence of the expected
247 bands at ≈1350 cm⁻¹ (D-band) and ≈1580 cm⁻¹ (G-band)
248 due to the defects and disorders, and the graphitized structure
249 of rGO, respectively ([Figure 2B](#)). The I_D/I_G intensity ratio was
250 found to be 0.93 for rGO. The PAA fibers did show a Raman
251 band at 1728 cm⁻¹ assigned to C=O functional groups,
252 whereas the bands at 1235 and 1285 cm⁻¹ are ascribed to
253 –CH₂– bonds.⁵⁰ In the case of PAA@rGO, the bands of rGO
254 are identifiable in the Raman spectrum. Additionally, Fourier
255 transform infrared (FTIR) and X-ray photoelectron spectroscopy
256 (XPS) spectra of PAA@rGO were obtained, where the
257 FTIR analysis did not provide any information about rGO
258 incorporation due to its low amount, but the presence of
259 delocalized π-electrons was evident in the XPS spectra (C 1s
260 scan) ([Figure S3](#)). 261

262 To ensure long-term stability in the aqueous media of the
263 hydrophobic PAA@rGO nanofibers, the fibers were cross-
264 linked to prevent their dissolution. As reported earlier, β-
265 cyclodextrin, a molecule containing multiple hydroxyl groups
266 can be efficiently used to cross-link the PAA polymer upon
267 heat treatment under vacuum for 30 min.⁵¹ The morphology
268 of the resulting cross-linked PAA@rGO nanofibers was probed
269 using scanning electron microscopy (SEM). [Figure 2C](#) shows
270 the formation of dense mats of interwoven nanofibers 400 ±
271 150 nm in diameter. Furthermore, the integration of rGO did
272 not alter the morphology of the nanofibers (see the Supporting
273 [Information](#), [Figure S1](#)). The stability of the cross-linked
274 PAA@rGO nanofibers was validated by immersing them into
275 aqueous solutions for several days at 37 °C. From the SEM
276 images in [Figure 2D](#), it is evident that the cross-linked PAA@
277 rGO nanofibers preserve their nanostructures and no
278 dissolution of the fiber networks occurs even after immersion
279 for days.

280 After fabrication, the photothermal properties of the PAA@
281 rGO nanofiber mats were explored ([Figure 3A](#)). Whereas PAA
282 displayed only a negligible photothermal heating, direct
283 irradiation of PAA@rGO (4 mg immersed in 1 mL PBS)
284 with a continuous wave laser at 980 nm (1 W cm⁻²) for 5 min
285 results in a surface temperature of about 67 ± 2 °C. Even
286 decreasing the laser power density to 0.5 W cm⁻² showed a

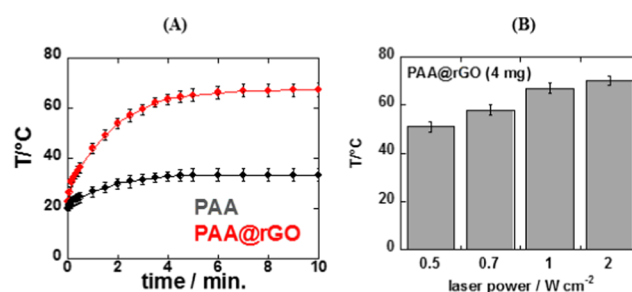
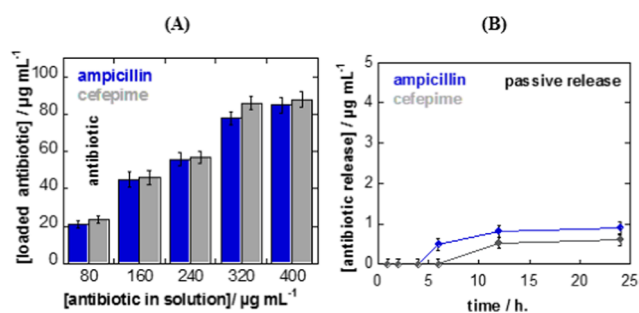


Figure 3. Photothermal properties of cross-linked PAA@rGO: (A) photothermal heating curves of PAA (gray) and PAA@rGO (red) nanofiber patches (4 mg) immersed in PBS (0.1 M, pH 7.4) and irradiated at 980 nm (1 W cm⁻²) for 10 min and (B) temperature profile of PAA@rGO nanofiber mats upon 10 min irradiation at varying power densities.

287 rapid increase in the surface temperature of PAA@rGO
288 nanofibers, reaching saturation at approximately $51 \pm 2^\circ\text{C}$
289 within 5 min in a wet environment. No melting of nanofibers
290 occurred under the used conditions, thus preserving the
291 structural integrity of the fibrous mats. Thus, with modulation
292 of laser power density, these nanofiber mats exhibited
293 controlled photothermal heating ability within a temperature
294 range from 20 to $67 \pm 2^\circ\text{C}$.

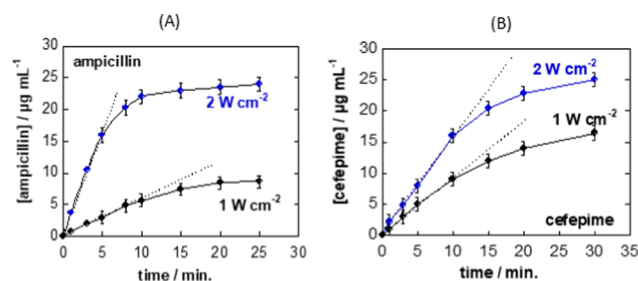
295 Loading and Release of Antibiotics into Nanofibers.

296 Integration of antibiotics into the cross-linked PAA@rGO
297 nanofiber mats was achieved by simply immersing the
298 nanofibers into the aqueous solution of ampicillin or cefepime
299 in water at 4°C for 72 h. Both antibiotics contain aromatic
300 structures together with amino and carboxylic acid functional
301 groups, thus promoting their interaction with rGO through
302 π - π stacking and/or electrostatic interactions. The loading
303 capacity of PAA@rGO nanofibers for AMP and cefepime was
304 determined from the amount of the antibiotic in the solution
305 before and after loading using HPLC. The loading capacity of
306 PAA@rGO for ampicillin and cefepime is comparable as seen
307 in Figure 4A. Using the solution concentration of $400 \mu\text{g mL}^{-1}$



308 of the antibiotics resulted in the loading of about $85 \pm 3 \mu\text{g}$
309 mL^{-1} (21%) and $89 \pm 3 \mu\text{g mL}^{-1}$ (22%) for ampicillin and
310 cefepime, respectively. Ampicillin has a $\text{p}K_{\text{a}} \approx 4$ –5 (COOH)
311 and $\text{p}K_{\text{a}} \approx 9$ (NH_2), making it zwitterionic at physiological
312 pH. A fair amount of the amine groups are positively ionized at
313 physiological pH and this promotes their interaction with the
314 PAA@rGO nanofiber mats ($\text{p}K_{\text{a}} \approx 4.5$).⁵² The quaternary
315 ammonium group of the *N*-methylpyrrolidine groups in
316 cefepime (Figure 1) makes the drug positively charged and
317 results in interaction with the ionized carboxylic acid groups in
318 PAA and negatively charged rGO that has a ζ -potential of -50
319 ± 2 mV. Due to their hydrophilic nature, it can be expected
320 that the nanofibers swell in aqueous media and thus load the
321 antibiotics efficiently. Remarkably, the passive release from the
322 antibiotic-loaded PAA@rGO nanofibers, PAA@rGO-AMP,
323 and PAA@rGO-CFP, was found to be negligible. For the first
324 5 h, the amount of antibiotic release was below the detection
325 limit of the HPLC analysis. After 24 h incubation in PBS (0.1
326 M, pH 7.4) about $0.8 \pm 0.1\%$ was released via passive diffusion
327 (Figure 4B).

328 Encouraged by the excellent stability of the PAA@rGO to
329 retain the loaded antibiotics, we explored the photothermal
330 properties of the PAA@rGO nanofiber mat to trigger the drug
331 release. Figure 5A shows the release profiles for ampicillin as



332 well as cefepime upon irradiating PAA@rGO nanofibers
333 loaded with ampicillin ($25 \mu\text{g mL}^{-1}$) and cefepime ($25 \mu\text{g}$
334 mL^{-1}) with NIR light. Using a laser power density of 2 W
335 cm^{-2} results in an almost complete release of ampicillin ($24 \mu\text{g}$
336 mL^{-1} ; $\approx 96\%$ of loaded ampicillin) with a release rate of $3.2 \mu\text{g}$
337 $\text{mL}^{-1} \text{min}^{-1}$, which can be lowered to $0.5 \mu\text{g mL}^{-1} \text{min}^{-1}$ using
338 a phototrigger of 1 W cm^{-2} . The released ampicillin
339 concentrations are much larger than the minimum inhibitory
340 concentration (MIC) value of ampicillin for Gram-negative
341 *Escherichia coli* K12 (Table 1). Ampicillin shows no activity for
342 *S. aureus* and *Staphylococcus epidermidis*.

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352 *Escherichia coli* K12 (Table 1). Ampicillin shows no activity for
353 *S. aureus* and *Staphylococcus epidermidis*.

354 **Table 1. Minimum Inhibitory Concentrations of Ampicillin and Cefepime for Different Bacteria Strains**

antibiotic	$\text{MIC}_{50}/\mu\text{g mL}^{-1}$ (<i>E. coli</i> K12)	$\text{MIC}_{50}/\mu\text{g mL}^{-1}$ (<i>S. aureus</i> ATCC 25923)	$\text{MIC}_{50}/\mu\text{g mL}^{-1}$ (<i>S. epidermidis</i>)
ampicillin	4.9 ± 0.5		
cefepime	1.1 ± 0.3	4.1 ± 0.8	10 ± 1.8

355 In the case of cefepime-loaded nanofiber mats, photothermal
356 activation of the fibers with 2 W cm^{-2} leads to a complete
357 release of the entrapped antibiotic ($25 \mu\text{g mL}^{-1}$) in a time
358 frame of ≈ 25 min, with a release rate of $1.36 \mu\text{g mL}^{-1} \text{min}^{-1}$
359 for cefepime for the first 10 min. The release rate can be tuned
360 upon using a lower power density: using a laser power of 1 W
361 cm^{-2} , the release rate of cefepime decreased to $0.91 \mu\text{g mL}^{-1}$
362 min^{-1} . Importantly, no burst release was observed in any of
363 these examples and the maximal amount released is about
364 twice of the MIC value for cefepime for *S. epidermidis* and
365 several times larger than the MIC value for cefepime for *E. coli*
366 K12 and *S. aureus* ATCC 25923 (Table 1).

367 **Antibacterial Activity under Passive and Active Release.** Although ampicillin has sufficient stability in the dry state, it is only short lived in a solution. Following photothermal-triggered ampicillin release, their antibiotic efficiency was assessed by determining the titer of viable bacteria able to grow. Figure 6A shows the change in OD_{600} values upon *E. coli* K12 incubation with different ampicillin-released samples at 1 W cm^{-2} . In agreement with the photothermal release profile (Figure 5A), about $2.5 \mu\text{g mL}^{-1}$ of ampicillin are released in the first minute, below the MIC_{50} value, whereas $6.8 \mu\text{g mL}^{-1}$ ampicillin (above the MIC_{50} value) was released after 3 min, resulting in the inhibition of *E. coli* growth. This underlines the fact that the photothermal-triggered ampicillin release does not effect the biological activity of the antibiotic.

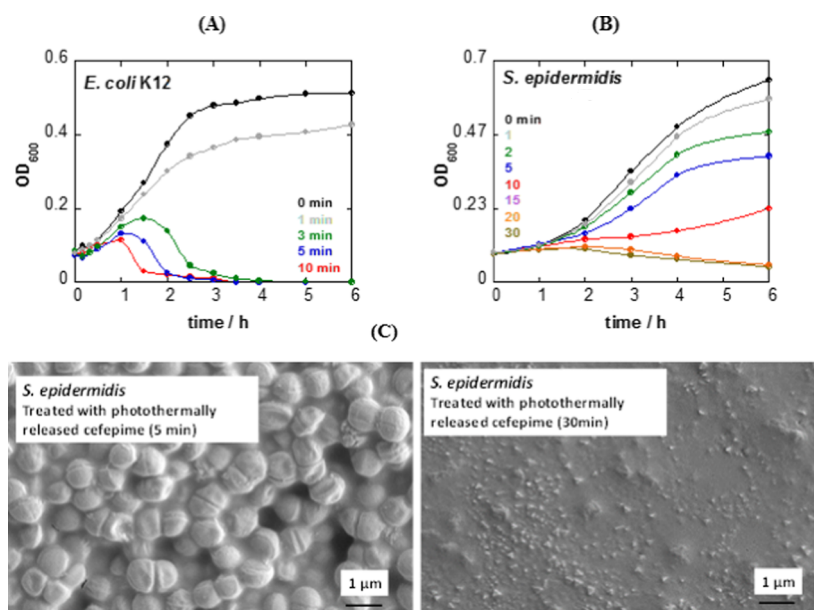


Figure 6. (A) Planktonic growth of *E. coli* K12 (1×10^8 cfu mL⁻¹) (A) without ampicillin (black, $t = 0$) and when treated with ampicillin released photothermally from PAA@rGO nanofiber mats at different time intervals corresponding to Figure 5A. (B) Planktonic growth of *S. epidermidis* without cefepime (black, $t = 0$) and when treated with cefepime released photothermally from PAA@rGO nanofiber mats at different time intervals corresponding to Figure 5B. (C) SEM images of *S. epidermidis* treated with cefepime photothermally released from PAA@rGO nanofiber mats for 5 and 30 min.

370 Whereas ampicillin is only effective toward Gram-negative *E.*
 371 *coli* K12, cefepime is active for Gram-negative and Gram-
 372 positive strains, with a higher MIC for *S. epidermidis* strain. *S.*
 373 *epidermidis* are known to be one of the most frequently
 374 encountered skin-colonizing bacteria and the predominant
 375 causes of nosocomial and community-associated skin in-
 376 fections. As expected, cefepime doses proved to be active
 377 against *S. epidermidis*. Cefepime released from cefepime-loaded
 378 nanofibers shows a release-dependent killing effect on *S.*
 379 *epidermidis* in accordance with the release profile determined in
 380 Figure 5B. Figure 6C shows the SEM images of *S. epidermidis*
 381 treated with photothermally released cefepime. Whereas the
 382 amount of cefepime is below the MIC value after 5 min and
 383 does not effect in any way the *S. epidermidis* growth, the
 384 pathogen is completely destroyed above the MIC value
 385 (corresponding to times >10 min). This indicates that the
 386 developed approach can be easily adapted for any desired
 387 antibiotic to eliminate a particular bacterial strain.

388 In addition to sustained antibiotic release, an important
 389 feature of any drug-release system is its reusability. Reloading
 390 of PAA@rGO with ampicillin or cefepime, as well as
 391 photothermally induced release could be attained with the
 392 similar efficiency over several cycles (Figure 7). Apart from just
 393 the possibility of reuse, the multiple loadings and release of
 394 antibiotics also demonstrate that the extent of upload and
 395 release is highly reproducible for these nanofibrous mats.

396 **Infectious Wound-Healing Studies Using rGO Nano-**
 397 **fibers.** There are different experimental models of skin
 398 infections that are caused by *S. aureus*.^{53,54} In our research,
 399 the bacteria were directly applied on the previously damaged
 400 skin. The tape-stripping approach, one of the most common
 401 models for the generation of superficial skin infection in
 402 mice,⁵⁵ was used here, as it allows to establish superficial
 403 infections in the epidermis, as well as the upper layer of the
 404 dermis. We monitored the skin infection progress in three
 405 animal groups: those treated with skin application of cefepime-

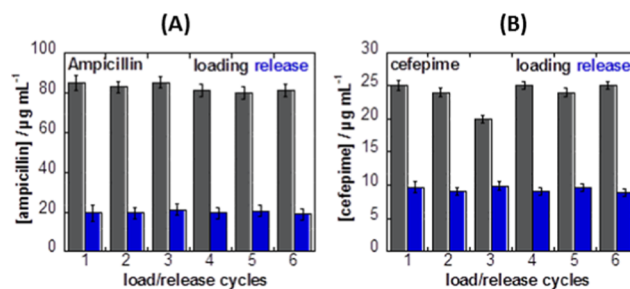


Figure 7. (A) Loading of ampicillin ($85 \mu\text{g mL}^{-1}$) and the amount of photothermally released ampicillin after 10 min laser irradiation (1 W cm^{-1}). (B) Loading of cefepime ($25 \mu\text{g mL}^{-1}$) and release of cefepime when activated at 980 nm at 2 W cm^{-2} .

406 loaded either PAA (PAA-CFP) or PAA@rGO (PAA@rGO- 406
 CFP) nanofiber mats and untreated mice (controls). The 407
 treatment in all groups started 24 h following the infection 408
 induction. Figure 8 displays images of the noninfected- and 409
 infected-wound groups untreated and treated with antibiotic- 410
 loaded nanofiber mats 48 h after infection initiation. The 411
 dressing (white arrows) was applied tightly to the wound area. 412
 The untreated areas and those treated with cefepime-loaded 413
 PAA nanofiber mats (PAA-CFP) and light activated for 10 min 414
 to release the antibiotics revealed inflammation and areas with 415
 abundant edema, erythema, and bacterial infiltration/pus. 416
 Blood vascularization and bacterial infiltration are in addition 417
 detected in the untreated groups and cefepime-loaded PAA 418
 fiber mats without embedded rGO (Figure 8B). This is in 419
 contrast to cefepime-loaded PAA@rGO mats, wherein the 420
 photothermal treatment prevented the bacteria from spreading 421
 (Figure 8A). Infected layers after 10 min illumination with a 422
 LED array through the antibiotic-loaded PAA@rGO nanofiber 423
 mats (PAA@rGO-CFP) show signs of resolution of inflam- 424
 mation (Figure 8A) and reduced blood vascularization and 425
 bacterial infiltration (Figure 8B). No damage to skin was 426

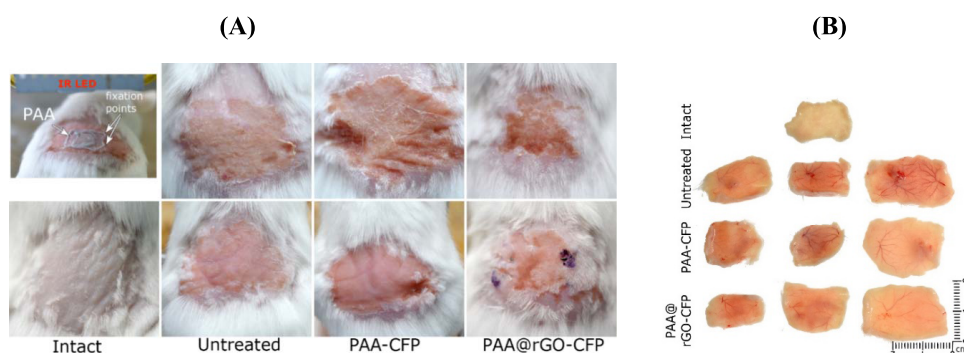


Figure 8. (A) Photographs of wound scars 24 h after treatment (980 nm , 1 W cm^{-2}) for 10 min with cefepime-loaded nanofibers mats (PAA-CFP; PAA@rGO-CFP). Negative control—intact skin; positive control—infected untreated skin. Insert at top left demonstrates the setup for animal treatment. (B) Photographs of intact mouse skin and three representative skin samples after 48 h of infection under different treatment conditions.

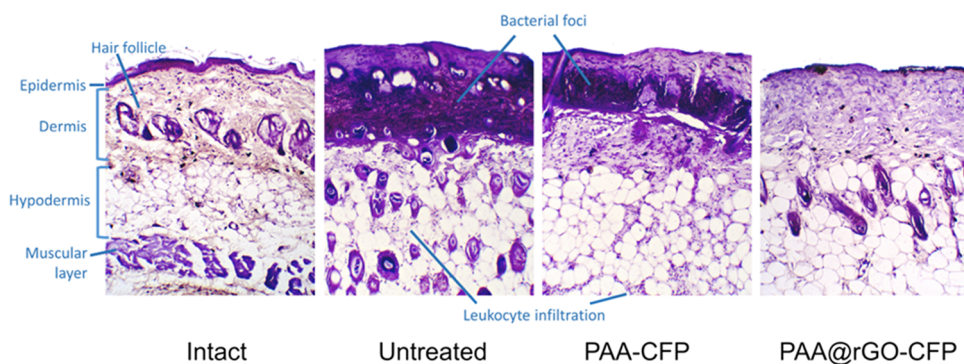


Figure 9. Histological examination of skin wound healing 48 h after the infection. Sections of skin tissues with Gram staining. Intact mouse skin; untreated skin (after infection); and photothermally treated infected skin part of the wound (PAA-CFP and PAA@rGO-CFP).

427 observed with LED illumination of the PAA@rGO nanofiber
428 patch during the in vivo experiments, as the skin surface
429 temperature was kept maximal at $52 \text{ }^\circ\text{C}$. This is opposed to
430 skin heating up to $67 \text{ }^\circ\text{C}$ when tissue damage could be
431 observed in meat samples (Figure S4).

432 Histological section of a representative skin specimen
433 (harvested at day 2 postinfection) after Gram staining (Figure
434 9) showed that *S. aureus* was superficially localized in the
435 epidermis and penetrated the dermis layers of untreated and
436 PAA-CFP skin samples. In addition, the leukocytic infiltration
437 was abundant in the deep layer of hypodermis, indicating
438 abundant bacterial presence. However, the infected wound
439 irradiated through the antibiotic-loaded PAA@rGO patch for
440 10 min indicated active wound healing without marks of
441 inflammation and bacterial foci. Epidermis revealed signs of
442 active regeneration. Thus, it could be concluded that the
443 topical application of PAA@rGO-CFP mats for photothermal
444 therapy exhibited the best wound-healing capability among
445 those tested.

446 ■ CONCLUSIONS

447 In summary, a novel nanofiber-based platform for “on-
448 demand” release of antibiotics is fabricated using rGO-
449 containing polymeric nanofibers. The rGO-embedded hydro-
450 philic nanofibers obtained using solution electrospinning are
451 loaded with two different antibiotics, namely, ampicillin and
452 cefepime. Although negligible release of antibiotic is observed
453 under physiological conditions, exposure to NIR irradiation
454 leads to release amounts sufficient to kill Gram-positive and
455 Gram-negative bacteria alike. A clear correlation between the
456 NIR-triggered antibiotic release and bactericidal activity is

457 observed. It can be anticipated that the facile fabrication and
458 modular nature of the construct reported here provides a
459 platform that can be adapted for on-demand delivery of various
460 drugs for combating different diseases. Although only two
461 specific antibiotics were chosen in this study, the platform is
462 amenable to loading of other antibiotics, either solo or in
463 combination to combat a plethora of bacterial infections.

464 ■ ASSOCIATED CONTENT

465 Supporting Information

466 The Supporting Information is available free of charge on the
467 ACS Publications website at DOI: 10.1021/acsami.8b14784.

468 SEM images of fabricated PAA nanofiber mats at
469 different scales, HPLC plot, and calibration curves of
470 antibiotic release studies, FTIR and XPS of PAA@rGO
471 and images of skin samples treated with NIR irradiation
472 (PDF)

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