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

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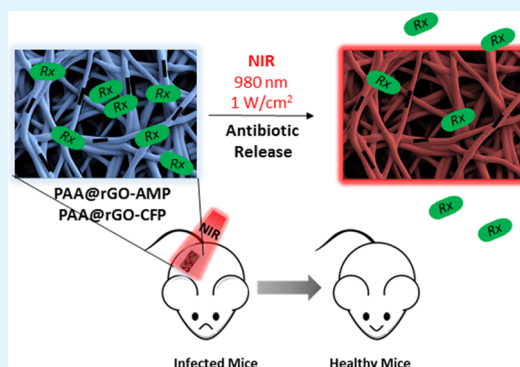
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## Supporting Information

**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

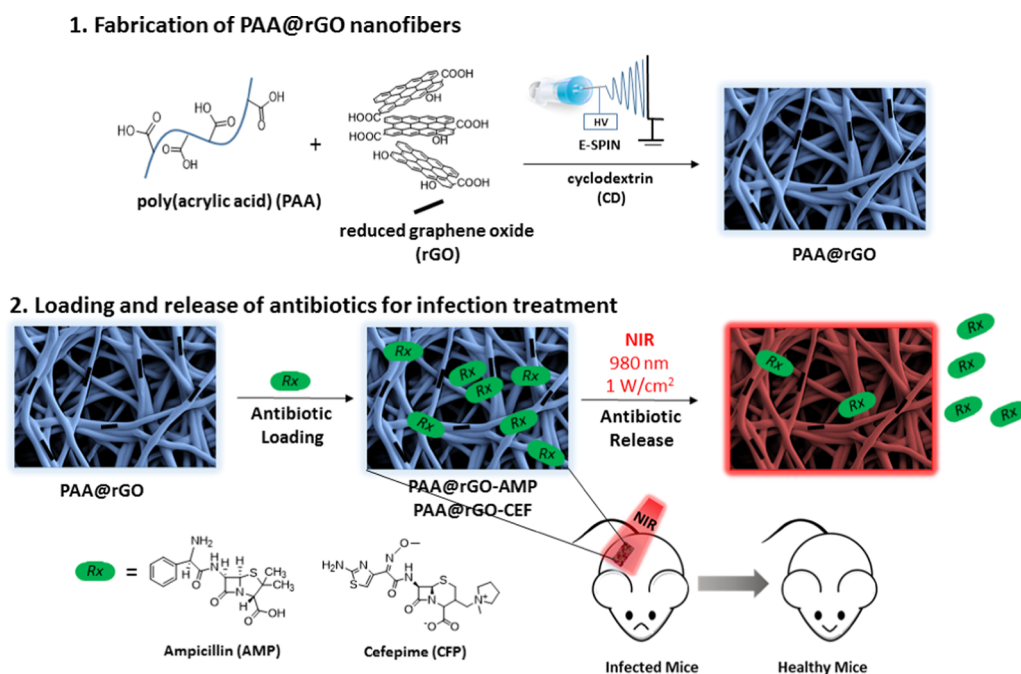
One of the challenges frequently encountered in traditional methods of drug delivery relates to poor control over proper dosage of therapeutic agent.<sup>1–3</sup> Maintaining the drug concentration within a particular therapeutic window and below toxic concentrations is challenging, and lack of efficient 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.<sup>4,5</sup> 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.<sup>6–9</sup>

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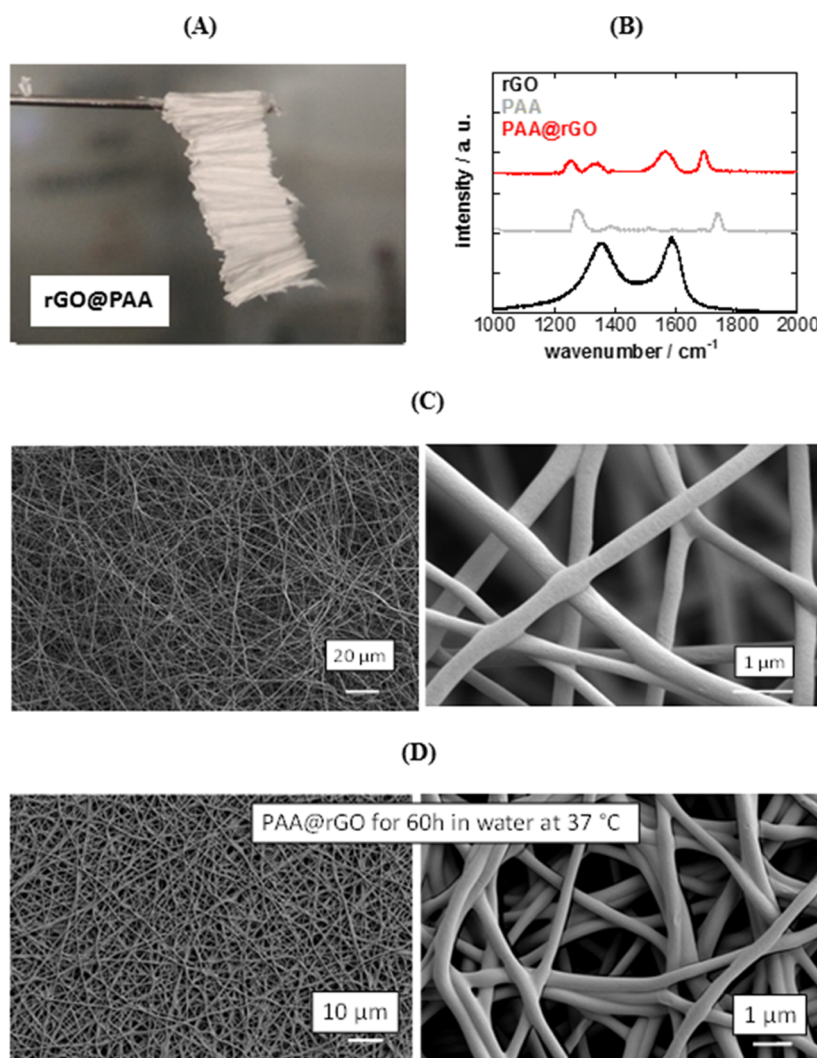
**Figure 1.** Illustration of the fabrication of antibiotic-loaded PAA@rGO nanofibers: (1) electrospinning of rGO-loaded PAA nanofiber mats followed by (2) loading with different antibiotics and photothermal-triggered antibiotic release.

Over the past decades, there is considerable evidence of the benefits polymeric materials as antibiotic delivery platform.<sup>10–12</sup> 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 antibiotics<sup>13</sup> to materials where polymers are chemically or physically assembled in the form of hydrogels,<sup>14–17</sup> micelles,<sup>18</sup> nanogels,<sup>19</sup> nanoparticles,<sup>20</sup> and nanofibers.<sup>21</sup> 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.<sup>1</sup> On the other hand, antibiotic-loaded bandages, foams, and dressings are preferential for external applications such for the management of chronic wounds.<sup>22,23</sup> 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.<sup>24</sup> 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.<sup>25</sup>

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.<sup>26–28</sup> This enables to select the most appropriate material for the intended application and enables tailoring of

drug release profiles.<sup>29</sup> 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.<sup>30</sup> 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.<sup>31,32</sup> 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.<sup>7</sup> Some photothermal active nanofibers formed through the incorporation of gold nanostructures,<sup>33–35</sup> carbon nanotubes,<sup>36,37</sup> carbon nanoparticles,<sup>38</sup> or more recently Cu<sub>2</sub>S<sup>39</sup> have been described. We have shown lately the interest in reduced graphene-oxide (rGO)-loaded hydrogels for photothermal-initiated insulin release.<sup>40</sup>

Interestingly, although reports of graphene oxide (GO) and rGO-containing nanofibers have emerged in recent years,<sup>41–44</sup> only one report used the photothermal properties of the resulting nanocomposite,<sup>45</sup> 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  $\beta$ -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  $67 \pm 2$   
 144 °C using a light source of 980 nm and  $1 \text{ W cm}^{-1}$  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  $\pi$ -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.<sup>46,47</sup> 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,  $\beta$ -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.<sup>40,48</sup> Briefly, GO aqueous suspension (5 mL, 0.5 mg mL<sup>-1</sup>) 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<sup>-1</sup>) 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<sup>-1</sup> 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<sup>-1</sup>) or  
202 cefepime (80–400 μg mL<sup>-1</sup>) 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<sup>-1</sup>) ([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<sup>-2</sup>) 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 previously<sup>49</sup> with  
222 some modifications. After removing the fur, a plaster was applied to  
223 the mouse skin (1 cm<sup>2</sup>) 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 × 10<sup>7</sup> cfu mL<sup>-1</sup>) 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 mm<sup>2</sup> in size, 8 W,  
232 2A, 940 nm), previously shown to be a safe condition for animals.<sup>49</sup>  
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.

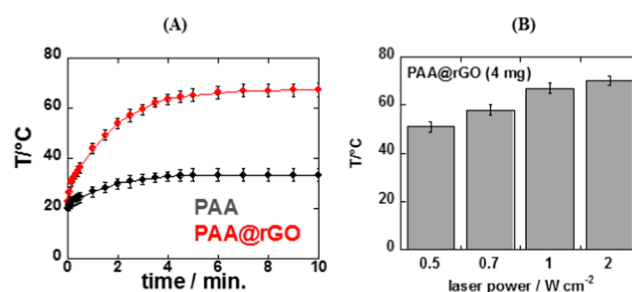
## 238 ■ RESULTS AND DISCUSSIONS

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<sup>-1</sup> (D-band) and ≈1580 cm<sup>-1</sup> (G-band)  
248 due to the defects and disorders, and the graphitized structure  
249 of rGO, respectively ([Figure 2B](#)). The I<sub>D</sub>/I<sub>G</sub> intensity ratio was  
250 found to be 0.93 for rGO. The PAA fibers did show a Raman  
251 band at 1728 cm<sup>-1</sup> assigned to C=O functional groups,  
252 whereas the bands at 1235 and 1285 cm<sup>-1</sup> are ascribed to  
253 –CH<sub>2</sub>– bonds.<sup>50</sup> 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

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

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

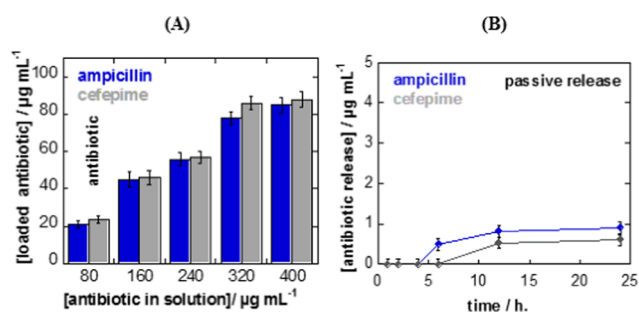


**Figure 3.** Photothermal properties of cross-linked PAA@rGO: (A) photothermal heating curves of PAA (gray) and PAA@rGO (red) nanofiber patches (4 mg) immersed in PBS (0.1 M, pH 7.4) and irradiated at 980 nm (1 W cm<sup>-2</sup>) for 10 min and (B) temperature profile of PAA@rGO nanofiber mats upon 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  $67 \pm 2^\circ\text{C}$ .

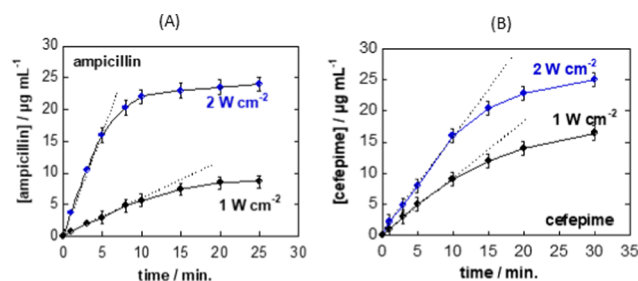
### 295 Loading and Release of Antibiotics into Nanofibers.

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  $4^\circ\text{C}$  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  $\pi$ - $\pi$  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  $\text{mL}^{-1}$  (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  $\text{p}K_{\text{a}} \approx 9$  ( $\text{NH}_2$ ), 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 ( $\text{p}K_{\text{a}} \approx 4.5$ ).<sup>52</sup> 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  $\zeta$ -potential of  $-50$   
319  $\pm 2$  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  $\text{mL}^{-1}$ ) with NIR light. Using a laser power density of  $2 \text{ W}$   
335  $\text{cm}^{-2}$  results in an almost complete release of ampicillin ( $24 \mu\text{g}$   
336  $\text{mL}^{-1}$ ;  $\approx 96\%$  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  $1 \text{ W cm}^{-2}$ . 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*.

343 well as cefepime upon irradiating PAA@rGO nanofibers  
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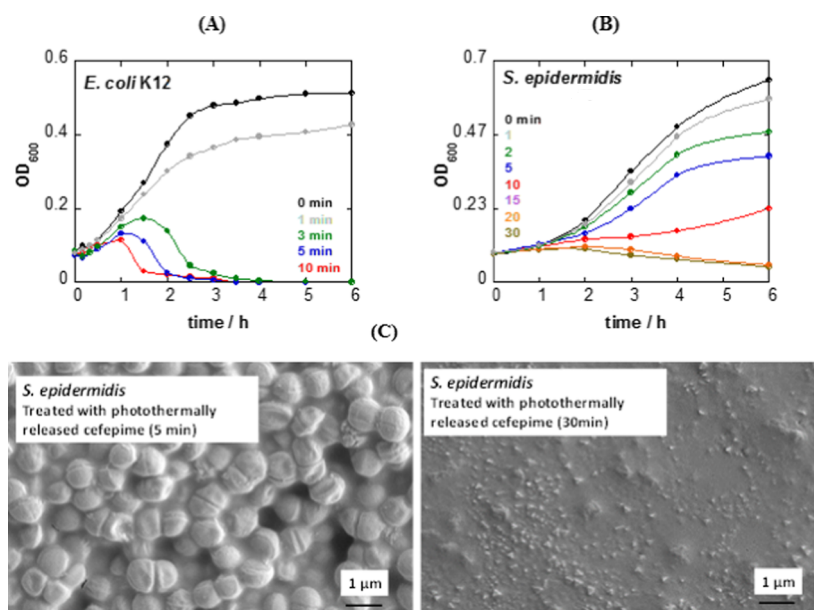
354 **Table 1. Minimum Inhibitory Concentrations of Ampicillin and Cefepime for Different Bacteria Strains**

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	$4.9 \pm 0.5$		
cefepime	$1.1 \pm 0.3$	$4.1 \pm 0.8$	$10 \pm 1.8$

355 In the case of cefepime-loaded nanofiber mats, photothermal  
356 activation of the fibers with  $2 \text{ W cm}^{-2}$  leads to a complete  
357 release of the entrapped antibiotic ( $25 \mu\text{g mL}^{-1}$ ) in a time  
358 frame of  $\approx 25$  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  $1 \text{ W}$   
361  $\text{cm}^{-2}$ , the release rate of cefepime decreased to  $0.91 \mu\text{g mL}^{-1}$   
362  $\text{min}^{-1}$ . 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

368 **Release.** Although ampicillin has sufficient stability in the  
369 dry state, it is only short lived in a solution. Following  
370 photothermal-triggered ampicillin release, their antibiotic  
371 efficiency was assessed by determining the titer of viable  
372 bacteria able to grow. Figure 6A shows the change in  $\text{OD}_{600}$   
373 values upon *E. coli* K12 incubation with different ampicillin-  
374 released samples at  $1 \text{ W cm}^{-2}$ . In agreement with the  
375 photothermal release profile (Figure 5A), about  $2.5 \mu\text{g mL}^{-1}$   
376 of ampicillin are released in the first minute, below the  $\text{MIC}_{50}$   
377 value, whereas  $6.8 \mu\text{g mL}^{-1}$  ampicillin (above the  $\text{MIC}_{50}$  value)  
378 was released after 3 min, resulting in the inhibition of *E. coli*  
379 growth. This underlines the fact that the photothermal-  
380 triggered ampicillin release does not effect the biological  
381 activity of the antibiotic.

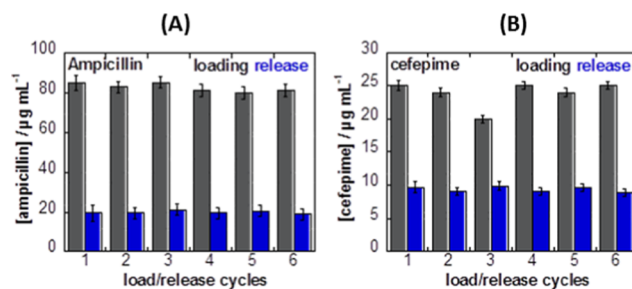


**Figure 6.** (A) Planktonic growth of *E. coli* K12 ( $1 \times 10^8$  cfu mL<sup>-1</sup>) (A) without ampicillin (black,  $t = 0$ ) and when treated with ampicillin released photothermally from PAA@rGO nanofiber mats at different time intervals corresponding to Figure 5A. (B) Planktonic growth of *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.

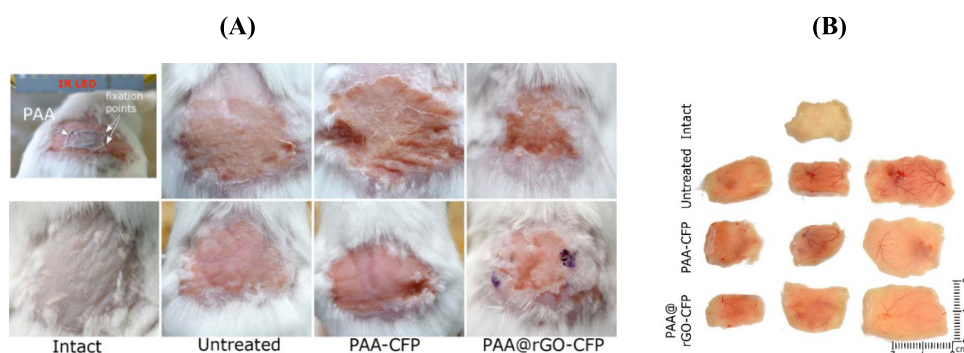
388 In addition to sustained antibiotic release, an important  
 389 feature of any drug-release system is its reusability. Reloading  
 390 of PAA@rGO with ampicillin or cefepime, as well as  
 391 photothermally induced release could be attained with the  
 392 similar efficiency over several cycles (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*.<sup>53,54</sup> 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,<sup>55</sup> 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-

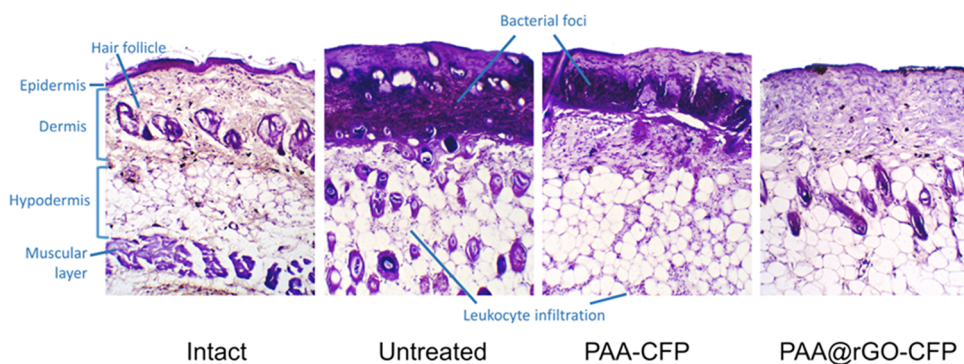


**Figure 7.** (A) Loading of ampicillin ( $85 \mu\text{g mL}^{-1}$ ) and the amount of photothermally released ampicillin after 10 min laser irradiation ( $1 \text{ W cm}^{-1}$ ). (B) Loading of cefepime ( $25 \mu\text{g mL}^{-1}$ ) and release of cefepime when activated at  $980 \text{ nm}$  at  $2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

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 ( $980\text{ nm}$ ,  $1\text{ W cm}^{-2}$ ) 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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