



Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutical Nanotechnology

Graphene Oxide–Based Nanocomposite for Sustained Release of Cephalexin

Nuwanthi P. Katuwavila¹, Yasuri Amarasekara², Vimukthi Jayaweera¹,
Chamil Rajaphaksha³, Chinthika Gunasekara⁴, Inoka C. Perera³,
Gehan A.J. Amaratunga^{1,2,5,6}, Laksiri Weerasinghe^{1,2,*}

¹ Sri Lanka Institute of Nanotechnology (SLINTEC), Mahenwatta, Pitipana, Homagama, Sri Lanka² SLINTEC Academy, Mahenwatta, Pitipana, Homagama, Sri Lanka³ Department of Zoology and Environment Sciences, Faculty of Science, University of Colombo, Colombo, Sri Lanka⁴ Department of Microbiology, Faculty of Medicine, University of Sri Jayewardenepura, Nugegoda, Sri Lanka⁵ Electrical Engineering Division, Department of Engineering, University of Cambridge, 9 JJ Thomson Avenue, Cambridge CB3 0FA, UK⁶ International Joint Research Center for Optoelectronic and Energy Materials, Yunnan University, Kunming 650091, China

ARTICLE INFO

Article history:

Received 13 June 2019

Revised 12 September 2019

Accepted 23 September 2019

Keywords:

graphene
drug delivery system(s)
nanodrug(s)
nanomedicine
nanocomposite(s)
nanoparticle(s)
nanotechnology
multidrug resistance
controlled release
drug resistance

ABSTRACT

A sustained-release carrier system for the drug cephalexin (CEF) using functionalized graphene oxide is reported. PEGylation of GO (GO-PEG) and successful loading of CEF into PEGylated graphene oxide (GO-PEG-CEF) nanoconjugate are confirmed by Fourier transform infrared spectroscopy, Raman spectroscopy, and thermogravimetric analysis. Encapsulation efficiency of 69% and a loading capacity of 19% are obtained with the optimized formulation of GO-PEG-CEF. *In vitro* CEF release profiles show an initial burst release followed by a more sustained release over a 96 h period with cumulative release of 80%. The half maximal inhibitory concentration (IC₅₀) values have both dose- and time-dependent antibacterial activity for GO-PEG-CEF against both gram-positive and gram-negative bacteria while pure CEF showed only dose-dependent antibacterial activity. The minimum inhibitory concentration values of GO-PEG-CEF are 7.8 and 3.9 µg/mL against *S. aureus* and *B. cereus*, respectively, while it is 10 µg/mL with pure CEF against both gram-positive bacteria. This confirms the enhanced antibacterial activity of GO-PEG-CEF over pure CEF against gram-positive bacteria. These findings therefore show GO-PEG-CEF is promising as a sustained-release nanoantibiotic system for effective treatment against *S. aureus* and *B. cereus* infections.

© 2019 Published by Elsevier Inc. on behalf of the American Pharmacists Association.

Introduction

The use of nanoparticulate carriers can allow for more efficient drug loading, targeted delivery, controlled release, and reduced toxicity of a drug.¹ So far, extensive research has been carried out on various types of nanomaterials such as liposomes, polymeric nanoparticles, dendrimers, and solid lipid nanoparticles in drug delivery applications.^{2,3} Recently, graphene oxide (GO), a 2-dimensional nanomaterial, has been explored as a novel drug carrier for the loading of various therapeutics including anti-cancer drugs, poorly soluble drugs, antibiotics, antibodies, peptides, DNA, and RNA.⁴⁻⁶ The use of graphene and graphene oxide for loading and releasing biologically relevant molecules has become an exciting research area in pharmaceutical sciences on account of its

fascinating physical and structural properties such as high specific surface area, enriched oxygen-containing groups, high biocompatibility, physiological stability, and cost-effectiveness.^{7,8} The atomically flat graphene matrix provides a noncovalent platform for biologically active molecules to link via hydrogen bonding, hydrophobicity, π - π stacking, and electrostatic interactions.⁹ Specifically, the enriched oxygen-containing functional groups on GO sheets could assist stronger covalent interaction with the drug molecules through chemical reactions while also increasing the aqueous dispersion. Preparation of multifunctional graphene nanomaterials and PEGylated (PEG = polyethylene glycol) GO⁷ as a nanocarrier has been intensively studied on the loading and the release of the anticancer drug doxorubicin (DOX), antidiabetic drug insulin,⁶ and flavonoids.¹⁰ Besides GO, numerous graphene-based composites fabricated with various types of nanoparticles have also been explored in the field of drug delivery.¹¹ Among these composites, the GO iron oxide (GO-IONP) nanoparticle has been exploited by number of researchers¹²⁻¹⁴ on account of its enhanced physical, chemical, thermal, mechanical, and biological properties.¹⁵

This article contains supplementary material available from the authors by request or via the Internet at <https://doi.org/10.1016/j.xphs.2019.09.022>.

* Correspondence to: Laksiri Weerasinghe (Telephone: +94114650553).

E-mail address: laksiriw@slintec.lk (L. Weerasinghe).

<https://doi.org/10.1016/j.xphs.2019.09.022>

0022-3549/© 2019 Published by Elsevier Inc. on behalf of the American Pharmacists Association.

Although the electronic, thermal, and mechanical properties of graphene and graphene-based materials have received most attention, their antibacterial properties have also been recognized.¹⁶ Notable contributions are reported, on the antibacterial activity of graphene and graphene oxide toward *Escherichia coli*,¹⁷ GO-Ag nanocomposites,¹⁸⁻²⁰ graphene and GO nanowalls,²¹ and reduced graphene oxide-TiO₂ thin films.²² However, the underlying antibacterial mechanism of GO is not fully understood but postulated based on its ability to enhance oxidative stress, ROS generation, DNA fragmentation, cell membrane damage, and electron transfer interactions with the microbial membrane.^{23,24}

After the discovery of penicillin, only limited classes of alternative antibiotics have been established. Most of them are slight variations of their initial form and once resistance is developed, an entire class of antibiotics can become unusable for treatment. The resistance in bacteria to antibiotics is on the rise and rapid differentiation in microbes has developed “super-bugs” that cause fatalities in all regions of the world. Fluctuating serum levels and abrupt termination of antibiotics are the main reasons behind antibiotic resistance. It is in this context that we explore graphene-derived antibacterial compounds with sustained activity as a potential route to mitigate antibiotic resistance.

As an alternative way to battle against bacterial drug resistance, antibiotic-nanoparticle combinations have been proposed.²⁵ However, studies on the property of sustained release of drugs with such materials are limited. Developing antibiotic-graphene oxide nanocomposites to synergistically enhance the antibacterial activity and prolong its activity is a novel approach to combat antibacterial resistance which we explore in this work. The enhancement of antibacterial activity of CEF, in combination with graphene oxide in the nanocomposite form, is examined. CEF is an orally active cephalosporin beta-lactam antibiotic with a broad spectrum of activity against gram-positive and some gram-negative bacteria. It is advocated for the treatment of infections of the upper and lower respiratory tract, genitourinary system, skin, and soft tissue infections. CEF has relatively low solubility in water and it is very unstable at physiological pH.²⁶ The usual parenteral or orally given cephalexin results in high peak blood levels but fall well below therapeutic concentrations before administration of the next dose.²⁷ To maintain the therapeutic level, drug administration has to be repeated 3-4 times per day to result in an effective therapy. Furthermore, the development of an antibacterial resistance mechanism to the group of cephalosporin either by not penetrating to its site of action inside the bacterial cell or by destroying by beta-lactamase enzymes before reaching its site of action decreases the bioavailability of oral CEF tablets.²⁸ Therefore, developing a novel controlled release system for CEF would result in enhanced concentration of the antimicrobial agent at the site of infection, better patient compliance, and more constant blood levels. Furthermore, the need for high doses of antibiotic to obtain the therapeutic effect can be avoided through controlled release. In the present study, we have examined the controlled release and the antibacterial activity of PEGylated GO loaded CEF (GO-PEG-CEF) nanocomposite, aiming to formulate a controlled release nano-platform to combat against antibacterial resistance.

Materials and Methods

Materials

CEF was provided by Gamma pharmaceuticals (Colombo, Sri Lanka). Snake skin dialysis tubing (MWCO 3500) was purchased from Thermo Scientific (Waltham, NY). Polyethylene glycol 4000 (PEG 4000) and dicyclohexylcarbodiimide were purchased from

Sigma-Aldrich Chemical Company (St. Louis, MO). All other reagents were used directly as received without further purification.

Synthesis of GO-PEG-CEF Composite

GO was prepared by a modified Hummer's method starting from Sri Lankan vein graphite (purity ~99%).²⁹ PEGylation of GO was done using a previously reported method.³⁰ The resulting product (GO-PEG) was purified by centrifugation at 14,000 rpm for 30 min and washed 3 times with deionized water and dried in a vacuum oven at 60°C overnight. Loading of CEF to GO-PEG was carried out by stirring different amounts of CEF (0.02 mg to 0.2 mg) overnight with GO-PEG at a GO concentration of 0.2 mg/mL in deionized water. Unloaded CEF was removed by filtration through a 30 kDa ultra centrifugal filter unit, and its concentration was measured by UV-VIS-NIR spectrometer at (264 nm). The resulting composite was washed several times with deionized water and ethanol.

Characterization

Fourier transform infrared measurements of GO, GO-PEG, and GO-PEG-CEF were carried out using a Bruker Vertex 80 IR spectrometer (Germany) at a resolution of 4 cm⁻¹ from 4000-400 cm⁻¹. Raman spectroscopic characterization was done with Bruker Senterra (Germany) excited at 532 nm laser line. Ultraviolet-visible-near infrared (UV-VIS-NIR) spectra were recorded using a UV-3600 (Shimadzu, UV-VIS-NIR, Japan). Thermal decomposition of GO, GO-PEG, and GO-PEG-cep were analyzed using a SDT Q600 thermogravimetric analyzer (TA Instruments) from 25°C to 1000°C using a ramp rate of 10°C/min in air.

Determination of Encapsulation Efficiency, Loading Capacity, and Release of Cephalexin

The amount of incorporated CEF in GO-PEG composite was determined by measuring the UV absorbance of CEF remaining in the supernatant (free CEF) after centrifugation of the reaction mixture, using the UV-visible spectrophotometer at 264 nm. Then the concentration was calculated from a calibration plot obtained for pure CEF. Percentage encapsulation efficiency was calculated as follows.

$$\% \text{Encapsulation efficiency} = \frac{(m1 - m2)}{m1} \times 100$$

$m1$ = Initial weight of drug added

$m2$ = Remaining weight of drug in the supernatant

$$\% \text{Loading capacity} = \frac{(m1 - m2)}{m}$$

m = final weight of the composite

The release characteristics of cephalexin from the GO-PEG-CEF composite were studied in phosphate-buffered saline (PBS) solution at neutral pH 7.4. The cephalexin-loaded nanocomposite was dispersed in 5.00 mL of PBS solution and trapped inside a dialysis membrane and then immersed in 25.00 mL of buffer solution at 37°C with mild agitation. Aliquots (3.00 mL) were withdrawn at pre-determined time intervals and their UV absorbance was measured at 264 nm using a UV-visible spectrophotometer. The release medium was refreshed with another 3.00 mL of medium after each withdrawal. All measurements were performed in triplicate. Using the

calibration plot, the concentrations were calculated and hence the cumulative release percentages determined.

In Vitro Antibacterial Activity

The prepared GO and GO-PEG-CEF were screened for their antibacterial activity against 2 gram-positive bacterial species (*Staphylococcus aureus* [ATCC 25923], *Bacillus cereus* [ATCC 11778]) and 2 gram-negative species (*Escherichia coli* [ATCC 25922], *Pseudomonas aeruginosa* [ATCC 27853]) cultured in Muller Hinton broth at 37°C. For all planktonic growth and biofilm assays yeast nitrogen base supplemented with 100 mM glucose, Muller Hinton broth was used as the culture media.

Half Maximal Inhibitory Concentration (IC50) Test

The IC50 values of GO, GO-PEG-CEF, and pure CEF were determined using a bacterial biofilm grown in a 96-well plate. Briefly, standard cell suspensions of *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were prepared in sterile PBS solution. Standard cell suspensions (100 µL) were inoculated in triplicate to sterile flat bottomed microtiter plates and the plates were incubated for 90 min at 37°C for initial adhesion. After the adhesion phase, the plates were washed twice with 200 µL/well sterile PBS solution to remove nonadherent cells and the wells were filled with 100 µL/well sterile culture media. Plates were incubated at 37°C up to 96 h. After the incubation period, biofilms were exposed to series of concentrations of GO, GO-PEG-CEF, and pure CEF (0.125-2.00 µg/mL) for 24 and 48 h. Cell viability was assessed by MTT assay, and the results were expressed as a mean value of 3 independent experiments. All the assays were performed in triplicate in 3 different experiments.

Minimum Inhibitory Concentration Test

Resazurin reduction assay was used to determine the lowest concentration of GO and GO-PEG-CEF that can inhibit the growth of each bacterial strain. Briefly, a volume of 100 µL of each cultured bacterial suspensions was inoculated in triplicate into a sterile, flat bottom, polystyrene 96-well microtiter plate. After the incubation, the cells were treated with a dilution series (250-1.25 µg/mL) of each test sample. After incubating at 37°C for 18 h, the color change was visually assessed and the minimum inhibitory concentration (MIC) values were interpreted as the point at which the color change occurred. All samples were examined in triplicate.

Results and Discussion

Fabrication of GO

Nanosized GO was obtained by ultrasonication of GO and then followed a simple method to functionalize GO to obtain a well-dispersed aqueous GO solution. In the first step, GO was obtained from graphite using a modified Hummer's method.³¹ Functionalization of GO with polyethylene glycol (PEG) to get a stable and well-dispersed solution in physiological solutions was achieved by a simple esterification of PEG with carboxylated GO. Initially, all the epoxides, ester groups, and hydroxyl groups of GO were converted to -COOH by mixing the GO with ClCH₂COOH under strong basic conditions according to the literature.³² Upon grafting PEG molecules onto the -COOH groups, the product GO-PEG was obtained with high solubility and stability in water and cellular solutions, which is desirable for biological applications. The drug (CEF) loading was achieved by simply stirring the drug with GO-PEG overnight. Any unbound drug was removed by ultracentrifugation. The UV-VIS spectrum of the resultant product was measured to obtain the encapsulation efficiency of CEF on GO-PEG. The

binding of CEF on GO-PEG is physisorption mainly via π - π stacking and hydrophobic interaction.⁸ To determine the saturation level of CEF loading onto GO-PEG, different amounts of CEF were added to GO-PEG solution at physiological pH. The formulation which has the GO:CEF ratio as 1:1 by weight had the highest encapsulation efficiency as $69 \pm 3.6\%$ with a loading capacity of $19 \pm 1.5\%$. Hence, this formulation was chosen for the following experiments.

Characterization of GO-PEG-CEF

Characteristic structural changes attributed to GO-PEG and GO-PEG-CEF were confirmed by Fourier transform infrared spectroscopy (Fig. 1).

The presence of oxygenated functional groups, -OH stretching (~ 3427 cm⁻¹) and C=O stretching (~ 1716 cm⁻¹) in GO (Fig. 1a), is in good agreement with previous work. The successful PEGylation was confirmed by shifting of the C=O stretching (1724 cm⁻¹) due to the formation of ester linkage -COO⁻ in the GO-PEG, Figure 1b. In the CEF (C₁₆H₁₇N₃O₄S) loaded spectrum, Figure 1c, the broad -OH band has appeared in 3 small bands (3556, 3480, and 3416 cm⁻¹) due to the appearance of stretching vibrations of -NH₂ group and -OH stretching. Furthermore, a shift of the -OH stretching (~ 3430 cm⁻¹) to 3416 cm⁻¹ may reflect the physical entrapment of CEF with GO.

In Figure 2a, the thermogram of GO highlights 3 significant thermal events corresponding to the evaporation of water (below 100°C), decomposition of oxygenated functional groups (100°C-200°C), and the bulk pyrolysis of carbon skeleton (300°C-500°C). A similar thermogram was obtained with GO-PEG in Figure 2b but the starting decomposition temperature of the composite has shifted to around 150°C and the weight loss occurred during 350°C-500°C has increased from 8% in GO to 14% in GO-PEG. The difference between these weight losses is likely due to the PEG being coupled to GO. In the thermogram of GO-PEG-CEF in Figure 2c, the decomposition starts at 65°C and has degradation steps as in GO and GO-PEG at 100°C-350°C and 350°C-500°C. In each degradation step, the loss of weight is low when compared to GO and GO-PEG. This is likely due to the bonding of CEF into GO-PEG composite. The extra bonds between CEF and the GO-PEG have increased the thermal stability of the composite and over 75% of the original weight remains even at 800°C. This enhancement in stability indicates that the CEF-loaded GO-PEG composite would be suitable for sustained/slow release.

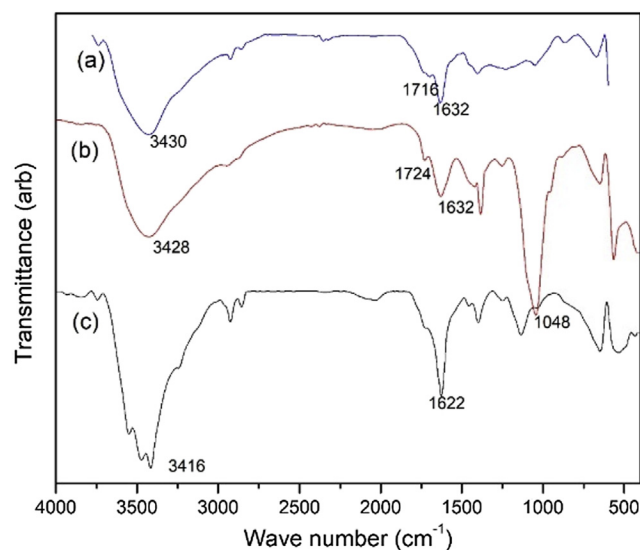


Figure 1. FTIR spectra of (a) GO, (b) GO-PEG, (c) GO-PEG-CEF where the GO-PEG-CEF was prepared using 1:1 weight ratio of GO to CEF.

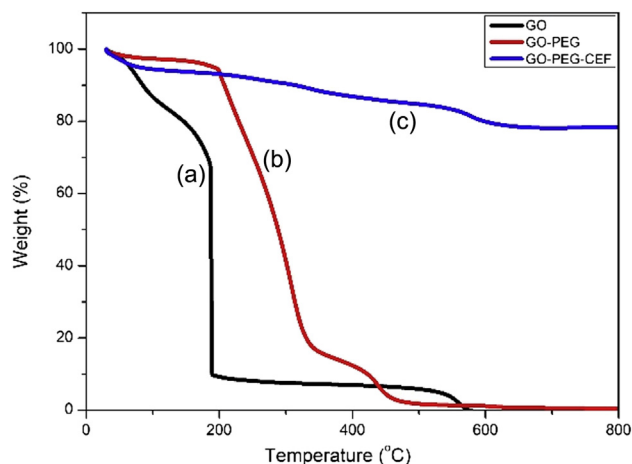


Figure 2. TGA thermograms of (a) GO, (b) GO-PEG, and (c) GO-PEG-CEF where the GO-PEG-CEF was prepared using 1:1 weight ratio of GO to CEF.

The Raman spectra of GO and GO-PEG, are shown in Figure 3. There are 2 characteristic peaks located at around 1380 and 1580 which are recognized as D and G bands of GO, respectively. The intensity ratios of the G band to the D band for GO and GO-PEG are 0.840 and 0.844, respectively. That GO-PEG and GO have similar I_D/I_G values which confirm that PEGylation does not destroy the aromatic structures of GO.

Drug Loading and In Vitro Release

The *in vitro* release profile of GO-PEG-CEF in PBS solution (pH 7.4) is shown in Figure 4. The drug release from the composite has 2 distinct phases. Initially, a burst release of about 45% in the first 6 h. This was followed by a slower exponential release of the remaining drug over another 90 h. The total release percentage was around 80%. The rapid initial release of CEF was probably due to the drug which was adsorbed at or on the surface of the GO. The delayed release may be attributed to the diffusion of the entrapped CEF in the GO interlayers. This release profile is consistent with a previous study in which vancomycin in solid lipid nanoparticles has a burst release over first 10 h followed by a sustained release for another

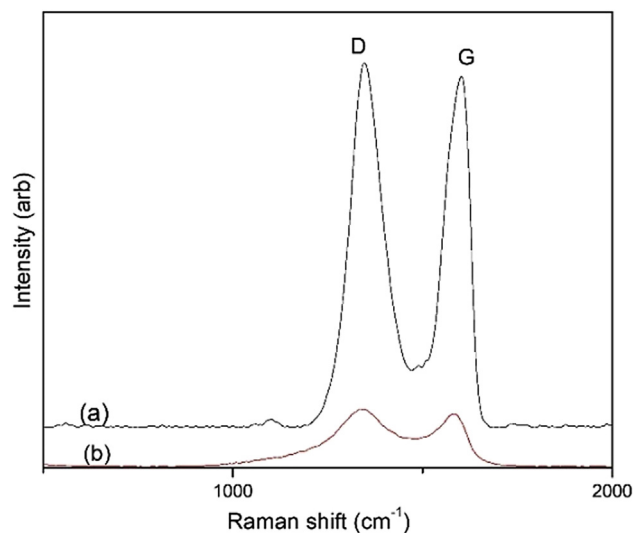


Figure 3. Raman spectra of (a) GO and (b) GO-PEG where Go was functionalized with PEG by a simple esterification.

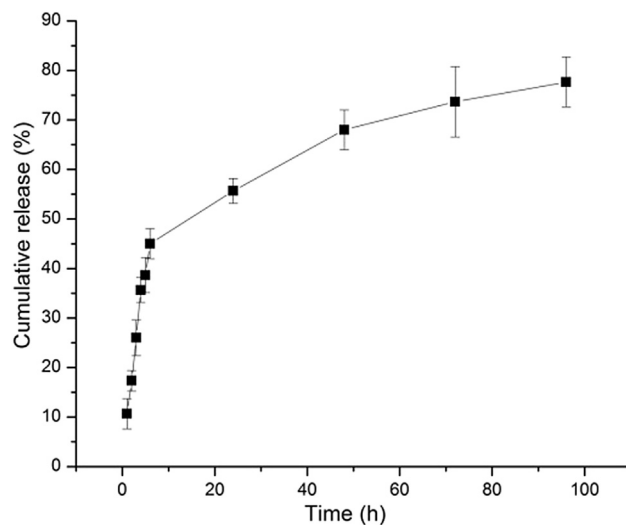


Figure 4. Cumulative release of CEF from GO-PEG-CEF at preselected time intervals in pH 7.4 buffer. Results were reported as mean \pm SD, $n = 3$.

62 h.³³ Another study of vancomycin in PLGA nanoparticles showed an initial burst release of about 10%-12% at the first sampling time followed by a slower exponential release of the remaining drug over the next 6-12 h.³⁴ Because of the shorter biological half-life of CEF, the preference is to use extended-release dosage forms. In literature, there were several findings reported on extended-release dosage forms of CEF. By using hydroxypropyl methyl cellulose as the matrix, the release of CEF has been retarded for 6 h and a twice-daily matrix tablet was formulated. Another sustained-release CEF tablet formulated with xanthan gum and sodium alginate polymers prolonged the CEF blood levels up to 8 h in humans.³⁵ But, in our study, GO-PEG-CEF composite has a significant improvement in prolonged releasing which can sustain up to 96 h.

In Vitro Antibacterial Activity

IC₅₀ and MIC tests were performed on 2 gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and 2 gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial strains to preliminarily investigate the antibacterial activity of GO and GO-PEG-CEF as a comparison to pure CEF.

Both GO and GO-PEG-CEF displayed a significant antibacterial activity against each of the strains when compared to the activity of pure CEF. GO itself has an antibacterial activity as previously reported.³⁶ In the composite GO-PEG-CEF, the IC₅₀ values against the growth of gram-positive bacterial strains are lower than that of the values of GO reflecting the combined effect of GO with the antibiotic CEF. The time-dependent antibacterial activity of the composite GO-PEG-CEF is confirmed by the lower IC₅₀ values obtained after 48 h of incubation (Table 1). This is due to the sustained

Table 1

IC₅₀ Values ($\mu\text{g/mL}$) of GO, GO-PEG-CEF, and CEF Obtained for Each Bacterial Strains at 24 and 48 h Incubation

| Bacterial Strain | GO | | GO-PEG-CEF | | CEF | |
|-------------------------------|------|------|------------|----------------|------|------|
| | 24 h | 48 h | 24 h | 48 h | 24 h | 48 h |
| <i>Staphylococcus aureus</i> | 1.7 | 0.31 | 0.24 | 0.17 | 0.15 | 0.08 |
| <i>Bacillus cereus</i> | 0.67 | 0.15 | 0.23 | 0.17 | 0.11 | 0.13 |
| <i>Escherichia coli</i> | 0.47 | 0.28 | 0.55 | — ^a | 0.21 | 0.20 |
| <i>Pseudomonas aeruginosa</i> | 0.24 | 0.22 | 0.22 | 0.12 | 0.26 | 0.21 |

Each value is a mean of 3 separate experiments.

^a Less than 50% of cell survival after 48 h.

Table 2
MIC Values ($\mu\text{g/mL}$) of GO and GO-PEG-CEF Obtained for Each Bacterial Strains After 18 h Incubation

| Composite | <i>Staphylococcus aureus</i> | <i>Bacillus cereus</i> | <i>Escherichia coli</i> | <i>Pseudomonas aeruginosa</i> |
|------------|------------------------------|------------------------|-------------------------|-------------------------------|
| GO | 62.5 | 31.2 | 31.2 | 250 |
| GO-PEG-CEF | 7.80 | 3.90 | 7.80 | 125 |
| CEF | 15.6 | 15.6 | 62.5 | 125 |

release of CEF from the GO-PEG-CEF with time. A similar study of vancomycin-loaded solid lipid nanoparticles showed an enhanced activity against *S. aureus* and methicillin-resistant *S. aureus* after 72 h incubation due to the extended release of vancomycin from the solid lipid nanoparticles. It is possible to observe the time-dependent activity of GO-PEG-CEF against gram-negative bacterial strains as well.

As shown in Table 2, the MIC values reflected the enhanced antibacterial activity of GO-PEG-CEF composite. The MIC values of the GO-PEG-CEF composite were 7.8 and 3.9 $\mu\text{g/mL}$ with *S. aureus* and *B. cereus*, respectively, whereas it was 10 $\mu\text{g/mL}$ for pure CEF with both bacterial strains. This reflects the synergistic effect of GO and CEF in GO-PEG-CEF composite. But such an observation could not be observed with gram-negative bacteria (Table 2). According to the literature, generally cephalosporin is more susceptible to gram-positive bacteria.³⁷ In a similar study, Manickam and co-workers have also been observed species-specific antibacterial effect of rGO-Ag nanocomposites.²⁰

When comparing the similar concentrations of pure CEF and GO-PEG-CEF, the actual concentration of CEF in the composite is much lower than that of pure CEF according to the loading capacity (19%). Thus, this composite can minimize the adverse effects of CEF due to the smaller doses of the drug. Furthermore, as it is proposed that GO can inhibit the bacterial growth by several mechanisms including oxidative stress, ROS generation, DNA fragmentation, cell membrane damage, and electron transfer interactions with the microbial membrane and CEF inhibits the synthesis of peptidoglycan in the bacterial cell wall, a nanoantibiotic with a dual mechanism of action against bacteria is obtained with the GO-PEG-CEF composite. These different mechanisms of action of GO and CEF as well as GO being a nanodelivery system which reduces the dosage of CEF required can make the development of bacterial resistance more difficult.

Conclusion

In this study, we investigate the potential of GO as an efficient system for sustaining the antibacterial activity of CEF. Upon successful encapsulation of CEF into GO, the anticipated sustained release of CEF was achieved. Moreover, the GO-PEG-CEF composite showed an enhanced antibacterial activity compared to positive control on gram-positive bacteria. GO-PEG-CEF could be an effective nano-based antibiotic system with synergistic antibacterial mechanisms to treat infections caused by gram-positive bacteria.

Associated Content

The following files are available free of charge. [Supporting information](#).

Acknowledgments

This work was funded by the Ministry of Science, Technology and Research, Sri Lanka (strategic project on graphene oxide). The authors wish to acknowledge Gamma Pharmaceuticals for providing pure API samples.

References

- Jong WH, Borm PJA. Drug delivery and nanoparticles: applications and hazards. *Int J Nanomedicine*. 2008;3(2):133-149.
- Iinuma H, Maruyama K, Okinaga K, et al. Intracellular targeting therapy of cisplatin-encapsulated transferrin-polyethylene glycol liposome on peritoneal dissemination of gastric cancer. *Int J Cancer*. 2002;99(1):130-137.
- Nanjwade BK, Bechra HM, Derkarc GK, Manvi FV, Nanjwade VK. Dendrimers: emerging polymers for drug-delivery systems. *Eur J Pharm Sci*. 2009;38(3):185-196.
- Yang K, Feng L, Shi X, Liu Z. Nano-graphene in biomedicine: theranostic applications. *Chem Soc Rev*. 2013;42(2):530-547.
- Pan Y, Sahoo NG, Li L. The application of graphene oxide in drug delivery. *Expert Opin Drug Deliv*. 2012;9(11):1365-1376.
- Turcheniuk K, Khanal M, Motorina A, et al. Insulin loaded iron magnetic nanoparticle-graphene oxide composites: synthesis, characterization and application for in vivo delivery of insulin. *RSC Adv*. 2014;4(2):865-875.
- Chen J, Liu H, Zhao C, et al. One-step reduction and PEGylation of graphene oxide for photothermally controlled drug delivery. *Biomaterials*. 2014;35(18):4986-4995.
- Sun X, Liu Z, Welsher K, et al. Nano-graphene oxide for cellular imaging and drug delivery. *Nano Res*. 2008;1(3):203-212.
- Liu J, Cui L, Losic D. Graphene and graphene oxide as new nanocarriers for drug delivery applications. *Acta Biomater*. 2013;9(12):9243-9257.
- Rahmanian N, Hamishehkar H, Dolatabadi JEN, Arsalani N. Nano graphene oxide: a novel carrier for oral delivery of flavonoids. *Colloids Surf B Biointerfaces*. 2014;123:331-338.
- Santhosh KK, Modak MD, Paik P. Graphene oxide for biomedical applications. *J Nanomed Res*. 2017;5(6):1-6.
- Liang J, Xu Y, Sui D, et al. Flexible, magnetic, and electrically conductive graphene/Fe₃O₄ paper and its application for magnetic-controlled switches. *J Phys Chem*. 2010;114(41):17465-17471.
- Ji L, Tan Z, Kuykendall TR, et al. Fe₃O₄ nanoparticle-integrated graphene sheets for high-performance half and full lithium ion cells. *Phys Chem Chem Phys*. 2011;13(15):7170-7177.
- Yang X, Zhang X, Ma Y, Huang Y, Wang Y, Chen Y. Superparamagnetic graphene oxide-Fe₃O₄ nanoparticles hybrid for controlled targeted drug carriers. *J Mater Chem*. 2009;19(18):2710-2714.
- Majewski P, Thierry B. Functionalized magnetite nanoparticles—synthesis, properties, and bio-applications. *Crit Rev Solid State*. 2007;32(3-4):203-215.
- Liu S, Zeng TH, Hofmann M, et al. Antibacterial activity of graphite, graphite oxide, graphene oxide, and reduced graphene oxide: membrane and oxidative stress. *ACS Nano*. 2011;5(9):6971-6980.
- Hu W, Peng C, Luo W, et al. Graphene-based antibacterial paper. *ACS Nano*. 2010;4(7):4317-4323.
- Zhang D, Liu X, Wang X. Green synthesis of graphene oxide sheets decorated by silver nanoprisms and their anti-bacterial properties. *J Inorg Biochem*. 2011;105(9):1181-1186.
- Huang L, Yang H, Zhang Y, Xiao W. Study on synthesis and antibacterial properties of Ag NPs/GO nanocomposites. *J Nanomater*. 2016;2016:1-9.
- Geetha BR, Muthoosamy K, Shipton FN, et al. The biogenic synthesis of a reduced graphene oxide-silver (RGO-Ag) nanocomposite and its dual applications as an antibacterial agent and cancer biomarker sensor. *RSC Adv*. 2016;6(43):36576-36587.
- Akhavan O, Ghaderi E. Toxicity of graphene and graphene oxide nanowalls against bacteria. *ACS Nano*. 2010;4(10):5731-5736.
- Akhavan O, Ghaderi E. Photocatalytic reduction of graphene oxide nanosheets on TiO₂ thin film for photoinactivation of bacteria in solar Light Irradiation. *J Phys Chem*. 2009;113(47):20214-20220.
- Li J, Wang G, Zhu H, et al. Antibacterial activity of large-area monolayer graphene film manipulated by charge transfer. *Sci Rep*. 2014;4:4359.
- Akhavan O, Ghaderi E, Akhavan A. Size-dependent genotoxicity of graphene nanoplatelets in human stem cells. *Biomaterials*. 2012;33(32):8017-8025.
- Allahverdiyev AM, Kon KV, Abamor ES, Bagirova M, Rafailovich M. Coping with antibiotic resistance: combining nanoparticles with antibiotics and other antimicrobial agents. *Expert Rev Anti Infect Ther*. 2011;9(11):1035-1052.
- Nightingale CH, Greene DS, Quintiliani R. Pharmacokinetics and clinical use of cephalosporin antibiotics. *J Pharm Sci*. 1975;64(12):1899-1927.
- Schneider H, Nightingale CH, Quintiliani R, Flanagan DR. Evaluation of an oral prolonged-release antibiotic formulation. *J Pharm Sci*. 1978;67(11):1620-1622.
- Caprile KA. The cephalosporin antimicrobial agents: a comprehensive review. *J Vet Pharmacol Ther*. 1988;11(1):1-32.
- Kumarathilaka P, Jayaweera V, Wijesekera H, Kottegoda IRM, Rosa SRD, Vithanage M. Insights into starch coated nanozero valent iron-graphene

- composite for Cr(VI) removal from aqueous medium. *J Nanomater.* 2016;2016:10.
30. Torbati BM, Ebrahimian M, Yousefi M, Shaabanzadeh M. GO-PEG as a drug nanocarrier and its antiproliferative effect on human cervical cancer cell line. *Artif Cells Nanomed Biotechnol.* 2017;45(3):568-573.
 31. Jayawardena VCP, Jayasundara DR, Amaratunga GA, Jayaweera V. *Method for the Synthesis of Graphene Oxide.* 2018. Patent US 20180029887.
 32. Xu H, Fan M, Elhissi AM, et al. PEGylated graphene oxide for tumor-targeted delivery of paclitaxel. *Nanomedicine.* 2015;10(8):1247-1262.
 33. Kalhapure RS, Mocktar C, Sikwal DR, et al. Ion pairing with linoleic acid simultaneously enhances encapsulation efficiency and antibacterial activity of vancomycin in solid lipid nanoparticles. *Colloids Surf B Biointerfaces.* 2014;117:303-311.
 34. Zakeri-Milani P, Loveymi BD, Jelvehgari M, Valizadeh H. The characteristics and improved intestinal permeability of vancomycin PLGA-nanoparticles as colloidal drug delivery system. *Colloids Surf B Biointerfaces.* 2013;103:174-181.
 35. Dhopeshwarkar V, O'Keeffe JC, Zatz JL, Deete R, Horton M. Development of an oral sustained-release antibiotic matrix tablet using in-vitro/in-vivo correlations. *Drug Dev Ind Pharm.* 1994;20(11):1851-1867.
 36. Singh R, Vinay S, Shrivastava R, et al. Synthesis, characterization and enhanced antimicrobial activity of reduced graphene oxide-zinc oxide nanocomposite. *Mater Res Express.* 2017;4(2):025401.
 37. Hsieh WC, Ho SW. Evaluation of antibacterial activities of cephalosporin antibiotics: cefazolin, cephaloridine, cephalothin, and cephalixin. *Chin J Microbiol.* 1975;8(1):1-11.