Colloids and Surfaces B: Biointerfaces 170 (2018) 347–354

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Enhanced antibacterial activity of carbon dots functionalized with ampicillin combined with visible light triggered photodynamic effects

Roxana Jijie[a,b], Alexandre Barras[a], Julie Bouckaert[c], Nicoleta Dumitrascu[b], Sabine Szunerits[a], Rabah Boukherroub[a,*]

[a] Univ. Lille, CNRS, Central Lille, ISEN, Univ. Valenciennes, UMR 8520, IEMN, F-59000 Lille, France
[b] Iasi Plasma Advanced Research Center (IPARC), Faculty of Physics, Alexandru Ioan Cuza University of Iasi, Bd. Carol I no. 11, Iasi, 700506, Romania
[c] Unité de Glycobiologie Structurale et Fonctionnelle (UGSF), UMR 8576 du CNRS et Université de Lille, 50 Av. de Halley, 59658 Villeneuve d'Ascq, France

ARTICLE INFO

Keywords:
Carbon dots
Ampicillin
Bactericidal activity
Escherichia coli
PDT

ABSTRACT

In the last years, carbon-based nanomaterials have attracted considerable attention in a wide range of fields, particularly in biomedicine, owing to their remarkable photo-physical and chemical properties. In this study, we demonstrate that amine-terminated carbon dots (CDs-NH_2) functionalized with ampicillin (AMP) offer a new perspective for antibacterial treatment. The amine-functionalized carbon dots were used as a carrier for immobilization and delivery of ampicillin (CDs-AMP) and as a visible light-triggered antibacterial material. Additionally, AMP immobilization on the CDs-NH_2 surface improves its stability in solution as compared to free AMP. The AMP conjugated CDs platform combines the antibacterial function of AMP and conserves the intrinsic theranostic properties of CDs-NH_2. Therefore, the AMP immobilized onto CDs-NH_2 surface together with the generation of moderate quantities of reactive oxygen species under visible light illumination are very effective to inactivate the growth of *Escherichia coli*.

1. Introduction

The increase of multidrug-resistant bacteria infections represents an important biomedical challenge, demanding the development of alternative antibacterial-based platforms for which pathogens will not be able to develop resistance. In some situations, even the aggressive antibiotic treatment is not able to eradicate the infection due to the ability of bacteria to form biofilms.

In recent years, antimicrobial nanoparticles (NPs) [1], nano-sized carriers for the delivery of therapeutic agents [2] or light-responsive NPs [3–5] offer new approaches to fight against infectious diseases. Among the various nanoparticles, fluorescent carbon dots (CDs) open promising avenues for bacteria detection, imaging and inactivation due their remarkable optical properties, their surface versatility and good biocompatibility [6–14]. For example, Mehta et al. [11] reported that CDs, synthesized by using *S. officinarum* juice at 120 °C, acted as excellent fluorescent probes for imaging of *Escherichia coli* (*E. coli*) bacteria. Amphiphilic CDs [7] and PEG-passivated CDs [12] revealed also their potential as fluorescent markers for bacterial detection and imaging. Zhong *et al.* [13] employed CDs modified with vancomycin for assaying *Staphylococcus aureus* (*S. aureus*). Dou et al. [14] showed that quaternary linear and branched polyethyleneimine passivated CDs exhibit promising antibacterial activity against both Gram-negative and Gram-positive bacteria.

To date, only limited attention has been paid to the use of fluorescent CDs as drug delivery vehicles [8,15–18]. This also applies to their application in photodynamic/photothermal (PDT/PTT) therapy and particularly when it comes to the treatment of bacterial infections. The advantage of antimicrobial PDT includes equal killing efficiency independent of antibiotic resistance, the repetition of therapy without cumulative toxicity, and high spatial control. There has been much effort in the design of effective platforms for the treatment of cancer using CDs in combination with conventional photosynthesizers (PS) such as protoporphyrin I [19] or zinc phthalocyanine [20], while less effort has been devoted to the intrinsic photodynamic and/or antibacterial properties of CDs. The effect of surface charge (negative, neutral and positive) of CDs on the growth of *Escherichia coli (E. coli)* and the mechanism of antibacterial activity induced by these carbon nanostructures was investigated by Bing and coworkers [21]. Upon 6 h incubation of *E. coli* with the CDs, it was found that the positively charged CDs exhibited the strongest bactericidal activity, while the negatively charged and uncharged CDs had almost no bactericidal activity. A mechanistic study revealed that the bacteria exhibited characteristic markers of apoptosis upon treatment with the positively and

* Corresponding author.
E-mail address: rabah.boukherroub@univ-lille1.fr (R. Boukherroub).

https://doi.org/10.1016/j.colsurfb.2018.06.040
Received 8 April 2018; Received in revised form 15 June 2018; Accepted 18 June 2018
Available online 19 June 2018

negatively charged CDs, similar to those encountered upon treatment with common antibiotics [21]. CDs, prepared from metronidazole (a wide spectrum antibiotic against obligate anaerobes, including *Peptostreptococcus micros*, *Prevotella intermedia, P. gingvivalis* and *Fusobacterium*) as the carbon source using hydrothermal conditions, displayed inhibition growth of *P. gingvivalis* [22]. Yang et al. [23] prepared quaternary ammonium functionalized CDs by conjugating lauryl betaine (BS-12) to amine-terminated CDs using the common NHS/EDC coupling chemistry. The developed material exhibited good fluorescence emission for simultaneous detection and inhibition of Gram-positive bacteria. Sattarahmady et al. [24] demonstrated that the bactericidal effect of CDs can be accelerated by near infrared (NIR, 808 nm) irradiation. The NIR irradiation caused an increase of the solution temperature, inducing ROS production and cell wall damage.

In the present study, we have focused on the design and characterization of multifunctional CDs and investigated their antibacterial activity. Till now, various starting materials and synthetic routes to prepare CDs have been developed. These methods can be classified as top-down [12,25–29] or bottom-up approaches [13,30,31]. Among them, the hydrothermal synthesis is mostly used, because it is a simple and efficient way to prepare CDs [6,25,32–36]. Recent studies have demonstrated that biocompatible multifunctional CDs may be prepared by using biological precursors [37–43], called green chemistry concept.

In this work, amine-functionalized carbon dots (CDs-NH_2) were synthesized by a simple hydrothermal treatment of citric acid and ethylenediamine. The primary amine groups on the CDs-NH_2 surface were further used for the covalent linking of ampicillin (AMP), a β-lactam antibiotic, to produce CDs-AMP nanostructures. The immobilization of AMP onto the CDs-NH_2 surface was supported by UV–vis spectrophotometry, Fourier transform infrared spectroscopy (FTIR), atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), thermogravimetric analysis (TGA), zeta potential and dynamic light scattering (DLS) measurements. The antibacterial activity of CDs-NH_2 and CDs-AMP conjugate with and without visible light illumination was evaluated using the *E. coli* K12 – MG 1655 strain by cell growth measurements, standard plate count method and fluorescence-based cell dead/live assay. Our results demonstrated the potential of CDs-AMP as an effective platform for bacteria eradication. The lowest concentration of AMP necessary to inhibit the growth of *E. coli* cells in the case of CDs-AMP conjugate (14 μg mL^{-1}) was improved compared to free AMP (25 μg mL^{-1}), indicating the advantage of the CDs-AMP conjugate. In addition, we demonstrated that exposure of CDs-AMP to visible light enhances its bactericidal activity. To the best of our knowledge, there are no reports on the use of a platform that combines the intrinsic photodynamic properties of CDs with the antibacterial function of antibiotics loaded on their surface to inhibit the growth of pathogens. The results from this study highlighted the enhanced stability of AMP loaded on the CDs as compared to free AMP in addition to increased antibacterial activity upon visible light irradiation. The existence of a large panel of antibiotics and precursors for CDs synthesis holds great promise for the development of multifunctional nanostructures for combating bacterial infections.

2. Experimental

2.1. Materials

Citric acid, ethylenediamine, 9,10-anthracenediylbis(methylene)dimalonic acid (ABDA), ampicillin (AMP), *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC·HCl), *N*-hydroxysuccinimide (NHS), acetic acid, sodium acetate, ninhydrin, potassium cyanide (KCN), pyridine, phenol, quinine sulfate, Hoechst 33342, and paraformaldehyde were purchased from Sigma-Aldrich and used as received.

2.2. Synthesis of amine-functionalized carbon dots (CDs-NH_2)

Amine-functionalized carbon dots were synthesized following a method similar to that reported by Zhu et al. [44]. In brief, citric acid (2.1 g) and ethylenediamine (670 μL) were dissolved in Milli-Q water (20 mL). Then the mixture was transferred into a Teflon-lined autoclave (125 mL acid digestion vessel no. 4748, Parr, France) and heated at 250 °C for 5 h. The resulting product was cooled to room temperature and dialyzed against Milli-Q water using a cellulose ester dialysis membrane for 3 days (Biotech CE N°131093, pore size 500–1000 Da) in order to remove unreacted small molecules. Then, dry mass of 200 μL solution was weighted by Sartorius microbalance (TG 209 F3 Tarsus, Netzsch). The yield was about 60% and the stock solution was stored at 4 °C.

2.3. Conjugation of ampicillin onto amine-functionalized carbon dots (CDs-AMP)

Ampicillin was dissolved in PBS at a concentration of 1 mg mL^{-1}. The carboxyl groups of ampicillin were activated with an equimolar of EDC·HCl and NHS for 30 min. CDs-NH_2 dissolved in PBS at a concentration of 1 mg mL^{-1} and the ampicillin solution were mixed at a volume ratio of 2/1 at room temperature overnight. After that, CDs-AMP conjugate solution was dialyzed (Biotech CE N°131093, pore size 500–1000 Da) against Milli-Q water to remove unreacted ampicillin and kept at 4 °C until use.

2.4. Characterization

Atomic force microscopy (AFM) measurements (NT-MDT Solver Pro-M type apparatus) were carried out in ambient air, in the tapping (non-contact) mode, using commercial standard silicon-nitride tip with a radius of approximately 10 nm (NT-MDT NGS01/Au). The sample was prepared by dropping an aqueous CDs-NH_2 suspension onto a silicon surface and dried at 37 °C.

Size and zeta-potential measurements were performed using a Zetasizer Nano-ZS (Malvern Instruments Inc. Worcestershire, UK). CDs-NH_2 were diluted to 10 μg mL^{-1} and measured in Milli-Q water at pH 7.0.

UV-Vis spectroscopic measurements were carried out using a Perkin Elmer Lambda UV/Vis 950 dual-beam spectrophotometer operating at a resolution of 1 nm. The UV–vis spectra were recorded in quartz cuvettes of 1 cm path length between 200 and 800 nm.

Emission fluorescence spectra were recorded between 220 and 800 nm using a Cary Eclipse spectrometer (Agilent, France). Solutions were excited from 300 to 400 nm in a 20 nm increment excitation (excitation and emission slit: 5 nm, scan rate: 600 nm/min).

Thermogravimetric analysis (TGA) was carried out on a TG 209 F3 Tarsus Netzsch in the temperature range of 30 to 980 °C using an Al_2O_3 crucible under nitrogen flow (20 mL min^{-1}) with a heating rate of 10 °C/min.

Fourier transform infrared (FTIR) spectra were recorded using a ThermoScientific FTIR instrument (Nicolet 8700) in the range between 650 and 4000 cm^{-1} at a spectral resolution of 6 cm^{-1}. 1 mg of dried CDs-NH_2 was mixed with 200 mg of KBr powder in an agar mortar. The mixture was pressed into a pellet under 7 tons of load for 2–4 min, and the spectrum was recorded immediately. A total of 64 accumulative scans were collected. The signal from a pure KBr pellet was subtracted as a background.

X-ray photoelectron spectroscopy (XPS) measurements were performed with a Theta Probe spectrometer (Thermo Fisher Scientific) spectrometer using a monochromatic Al Kα X-ray source (1486.6 eV) under a vacuum of about 2×10^{-6} Pa at a photoelectron take-off angle of 45°.

SEM images of pathogens were recorded using a Zeiss Compat Merlin instrument and secondary electron detector at 2 and 5 kV under high vacuum. The biological samples were fixed with 1%