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Editors:

Radim Cerkal

Natálie Březinová Belcredi

Lenka Prokešová

Aneta Pilátová

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Biogenic amines modified carbon quantum dots as antibacterial agent

Milica Gagic[1,2], Silvia Kociova[1,2], Lukas Richtera[1,2], Kristyna Smerkova[1,2], Vedran Milosavljevic[1,2]
[1]Department of Chemistry and Biochemistry
Mendel University in Brno
Zemedelska 1, 613 00 Brno
[2]Central European Institute of Technology
Brno University of Technology
Purkynova 123, 612 00 Brno
CZECH REPUBLIC

qqgagic@mendelu.cz

Abstract: An alternative to conventional way is needed to treat multiple drug resistant bacteria. In this work, four different amine modified carbon quantum dots (CQDs) were obtained by microwave irradiation treatment. The four different biogenic amines (spermidine, putrescine, cadaverine, and histamine) as capping agent and citric acid as a carbon precursor were used. Prepared CQDs were evaluated for their antibacterial activity against three common pathogenic bacteria (*Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus*), and the growth curves were modeled. The CQDs showed a strong broad-spectrum antibacterial activity. The bactericidal activity was linked to their specific surface chemistry and caused bacterial death, which was due to the electrostatic interactions between protonated CQDs and the lipids of the bacterial cell membrane. The biocompatibility of CQDs was tested in HBL100 and HEK293 cell lines and low or absence of toxicity was indicated.

Key Words: carbon quantum dots, antibacterial, biogenic amines, pathogenic bacteria, resistance

INTRODUCTION

Due to the systematic massive use of antibiotics, bacteria have evolved sophisticated mechanisms of drug resistance to avoid killing by antimicrobial molecules. Resistance is usually achieved through multiple biochemical pathways, and one bacterial cell may be capable of using a cadre of mechanisms of resistance to survive the effect of an antibiotic. It is estimated that bacteria resistant to antibiotics kill around 700,000 people per year. World Health Organization warns that there is an urgent need to invest in the research about resistant infections and way, how to treat them. The past decade has witnessed a substantial increase in the global use of nanoscale materials as innovative tools for combating these pathogens. Among the most recent discovered are carbon quantum dots (CQDs). CQDs are small carbon nanoparticles, generally exhibiting sizes smaller than 10 nm (Lim et al. 2015). They can be synthesized from any carbonaceous precursors, without being in general toxic, therefore they have been increasingly used in biological and biomedical applications (Zuo et al. 2016).

CQDs are reported by several studies as effective antibacterial agents. Although applied against both gram-positive and gram-negative bacteria, mode of action is not yet fully elucidated. Up to now, the antibacterial mechanism has been partially considered as the effect of disrupting the membrane integrity (Li et al. 2011). In 2016, Yang et al. reported CQDs with a minimum inhibitory concentration of 8 μg/mL for *Staphylococcus aureus* (Yang et al. 2016). The bactericidal activity was accomplished through electrostatic interaction between the anionic bacterial membrane and cationic residues on the CQDs surface (Liu et al. 2017). Similar electrostatically based inhibitory effect was described year after by Liu et al. against *Porphyromonas gingivali* (Liu et al. 2017). Depending on surface functionalization, CQDs may carry a negative, neutral or positive charge. To explore enhanced antibacterial effect based on electrostatic interaction between CQDs and bacteria, four biogenic amines (putrescine, spermidine, histamine, and cadaverine) were used as capping agents for CQDs (PCQDs, SCQDs, HCQDs and CCQDs). At physiologic pH these amines carry a net positive charge. It was demonstrated significant bactericidal activity of prepared CQDs toward three pathogenic bacteria.

The biocompatible CQDs were prepared through microwave irradiation of a mixture of citric acid and respective biogenic amine. The size and surface charge were characterized by dynamic light scattering.

MATERIAL AND METHODS

Chemicals

The chemicals used in this work were purchased from Sigma Aldrich (St. Louis, MO, USA). Deionized water was purified to produce 18.2 MΩ/cm MilliQ water using Millipore RG (Bedford, MA, USA).

Preparation of amine modified CQDs

For the preparation of CQDs, citric acid was used as the carbon source. 5.0 g of citric acid was mixed with 1.0 g of respective biogenic amine and diluted in 10 mL water. Then, 1.0 mL of the solution was aliquoted into the vials and heated at 110 °C and 300 W for 10 min (ramping time of 10 min) under microwave irradiation (Multiwave 3000; Anton-Paar GmbH, Graz, Austria). Finally, the products were purified by dialyzing (membrane with 1000 MWCO) against MiliQ, freeze dried and dissolved in water to obtain the concentration of 1.0 mg/mL.

Characterization of CQDs

The zeta potential (ζ) and size of CQDs were determined by dynamic light scattering technique (DLS) using the Zetasizer Nano ZS instrument (Malvern Instrument Ltd, UK). The parameters of particle size measurements were follows: refraction index of the dispersive phase of 3.00 and 1.333 for the dispersive environment, adsorption coefficient 10^{-3}, temperature 25 °C, equilibration time 120 s, measurement angle of 173° backscatter. For measurement, disposable cuvettes type ZEN 0040 was used, containing 50 µL of sample. The ζ measurements were also performed at 25 ºC in polycarbonate folded capillary cells, incorporated with Au plated beryllium-copper electrodes (DTS1070) and deionized H_2O was the dispersion medium. ζ were automatically obtained by the software using the Smoluchowski approximation.

In vitro bacterial testing

The antibacterial effect of CQDs was analyzed by bacterial growth curves. *Escherichia coli* NCTC 13216, *Klebsiella pneumonia* NCTC 8511, and *Methicillin resistant S. aureus* CCM 7110 (Czech Collection of Microorganisms, Brno, Czech Republic) were cultured on Muller-Hinton (MH) agar (Oxoid, Hampshire, UK) overnight at 37 °C.

Growth curve method

One hundred µL of each bacterial suspension (1.0×10^6 CFU/mL) was placed into a 100-well microplate and mixed with CQDs (1.0 mg/mL), in the ratio 1:1. To monitor growth trends, Bioscreen C (Oy Growth Curves Ab Ltd, Helsinki, Finland) was used. The bacterial growth curves were achieved by measuring the optical density (OD) of the cultures and plotting it against time. OD reads at 600 nm were monitored at time zero, and then at 30 min intervals for 24 h at 37 °C.

Cell viability assay

To assess effect of particles on the viability of HBL100 and HEK293 cell lines, MTT assay was performed. The cells viability was tested by Infinite 200 PRO (Tecan, Maennedorf, Switzerland) using 96 well microtiter plates. Each microtiter plate well was filled with 50 µL of medium containing the suspension of 5.000 cells. The cell lines were treated with the samples in wide concentration range (from 0 to 500 µg/mL) and incubated for 24 h at 37 °C with 5% CO_2.

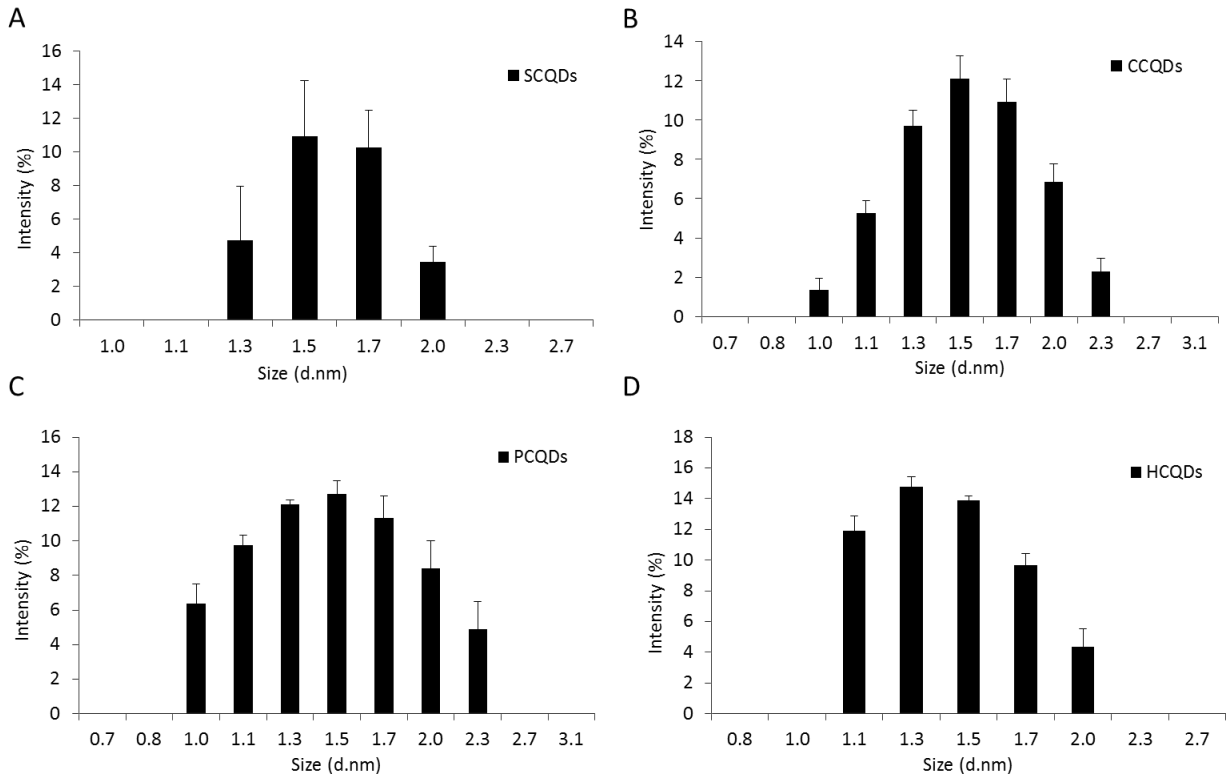
RESULT AND DISCUSSION

Synthesis and Characterization of CDs

In our experiment CQDs were synthesized by microwave irradiation. Firstly size and ζ potential were monitored by DLS. The hydrodynamic diameter size of prepared CQDs is presented in the Figure 1, which reveals that prepared QDs have size of approximately 1.5 nm and are very close

to each other. The functional groups on the surface are expect to have an effect on the overall charge of our CQDs. The ζ potential values measured were 1.96 ± 0.27, 0.22 ± 0.16, 1.25 ± 0.51, 1.40 ± 0.34, for CCQDs, PCQDs, SCQDs and HCQDs (Figure 1A–D), respectively. Zeta potential in all cases is positive thus indicating the predominance of amine groups on the dots surfaces.

Figure 1 The hydrodynamic size distribution of: A) spremidine carbon quantum dots, SCQDs B) cadaverine carbon quantum dots, CCQDs, putrescine carbon quantum dots, PCQDs and D) histamine carbon quantum dots, HCQDs. The error bars are values expressed as mean ± standard deviation.



In vitro antibacterial activity

Assuming that the OD at 600 nm reflects the number density of bacterial cells, its value was found to increase in a sigmoid fashion in the absence of prepared quantum dots. *In vitro* antibacterial effect of prepared CQDs was verified by bacterial growth curves and the results are shown in Figure 2. Growth patterns of bacteria *K. pneumonia, Methicillin resistant S. aureus* and *E.coli* were obtained by plotting bacteria inhibition values against time. Growth patterns of all the selected bacteria were obtained in the presence of different CQDs concentration (0–4 mg/mL) in the bacterial growth media. Growth inhibition of each bacteria was observed suggesting significant antibacterial effect of all applied CQDs with 100% efficiency at different concentration.

Toxicity study in mammalian cells

The biocompatibility and cytotoxic effect of the prepared nanomaterials on HBL-100 and HEK-293 cells were metabolically quantified through MTT assay. The obtained results were plotted as percentage cell viability versus concentration of CQDs and are shown in Figure 3. As can be inferred from the Figure 3, among all the prepared samples, the minimum percentage cell viability observed was around 60% corresponding to the highest tested concentration (2.5 mg/mL) of HCQDs. More than 80% of the HBL100 cells (Figure 3A), were viable after the treatment with 1.25 mg/mL and less in all the prepared samples. Based on the obtained results, going by the definition of biocompatibility which requires minimum 80% of cells to be viable, we can easily say that all our tested samples are biocompatible. On exposure to HEK293 cells (Figure 3B), prepared CQDs didn't show any decrease in cell viability at concentration from 2.5–0.03 mg/mL, thus indicating extraordinary biocompatibility.

Figure 2 The inhibition effect of prepared CQDs on (A) Staphylococcus aureus, (B) Escherichia coli and (C) Klebsiella pneumonia, respectively

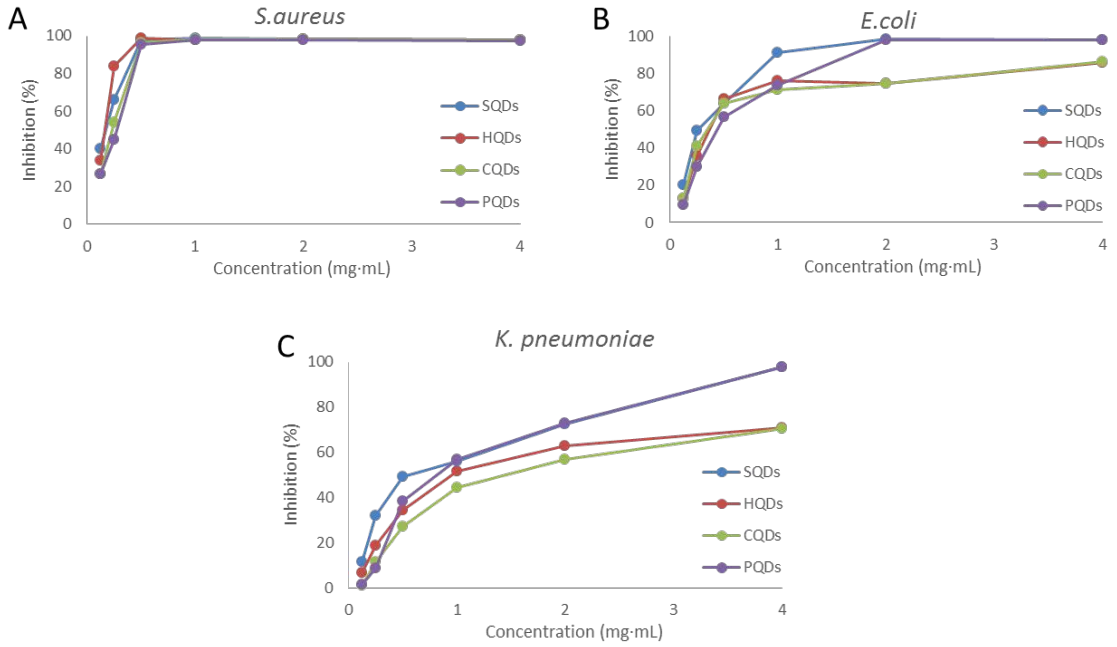
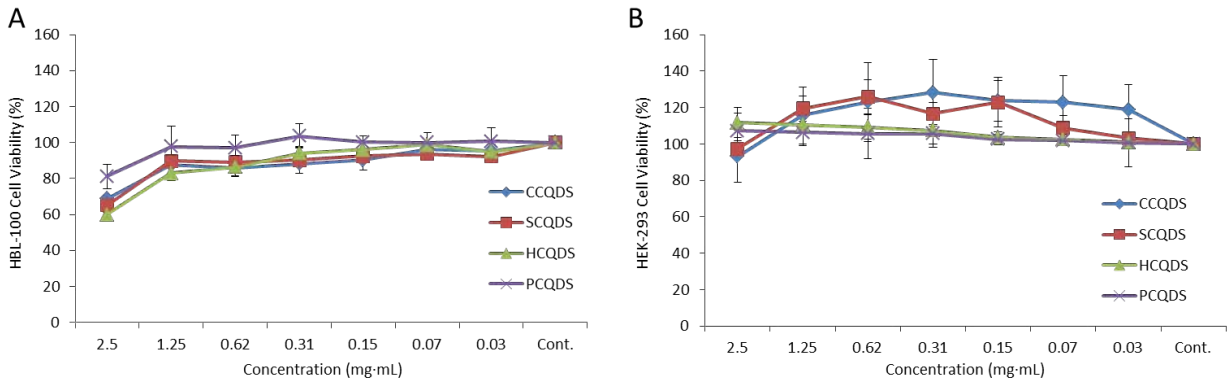


Figure 3 Cytotoxicity of prepared CQDs in (A) HBL-100 and (B) HEK-293 cells



CONCLUSION

In conclusion, we have demonstrated, through the growth curves analysis method that carbon quantum dots, modified with biogenic amines obtained by a simple microwave irradiation approach, have a capability to act as bactericides against tested strains. We found that all four CQDs formulations inhibited bacterial growth *in vitro* of both, gram-negative and gram-positive bacteria. Probably, the bacterial cell death induced by CQDs involved electrostatic interactions between the protonated forms of the nitrogen functional groups and the negatively charged cell membrane.

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