Photoactive carbogenic nanotracers with remarkable antimicrobial properties for pH-sensing applications

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INTRODUCTION

Carbogenic nanoparticles (also known as C-dots) constitute a new class of carbon-based materials, which can be easily synthesized via thermal treatment of carbon-rich precursors. These spherical nanotracers are composed of an amorphous core with an approximate size of below 10 nm (*Fig.1A*) and exhibit exquisite biocompatibility, simplicity of surface modification, excellent chemical stability and broad excitation spectra (*Fig.1B*) [1,2]. Their exceptional photoluminescent properties are related to the dual emissive mode with the excitation wavelength independent or dependent emission (*Fig.1B*), attributed to the presence of organic fluorophores or carbogenic cores, respectively [3].

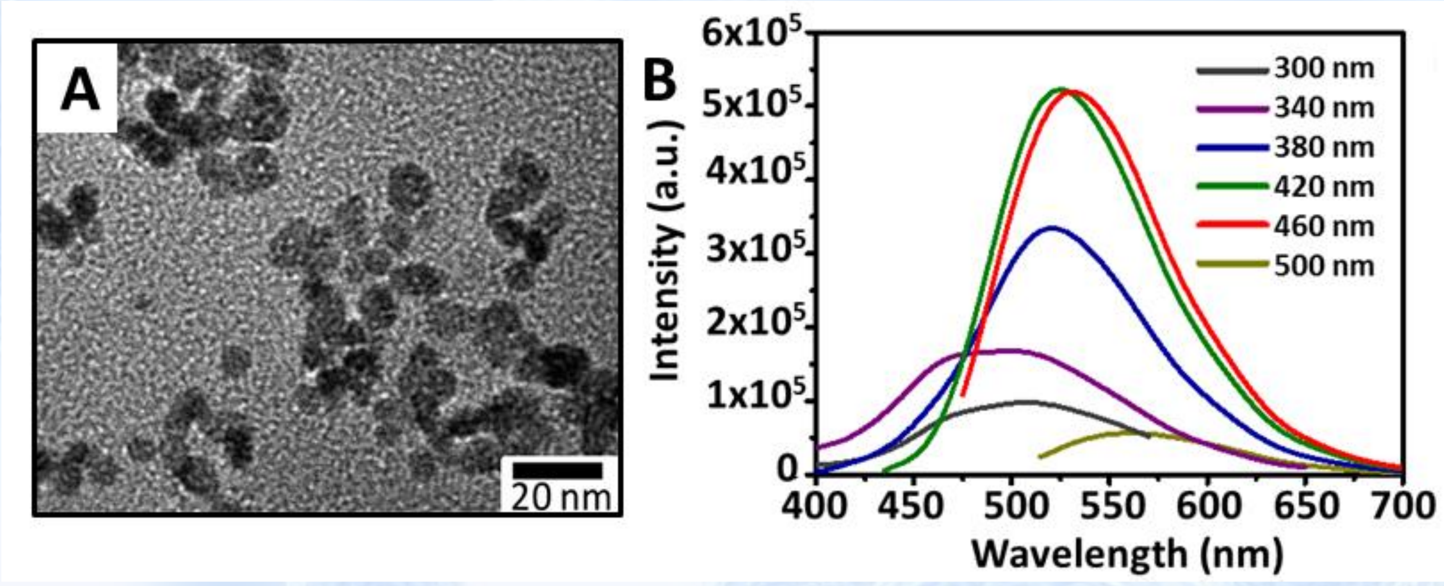


Figure 1. TEM image (A) together with the PL spectra of C-dots aqueous dispersion (B) recorded at excitation wavelengths ranging from 300 nm to 500 nm, with constant increment of 40 nm.

FLUORESCENT SENSORS

The monitoring of intercellular pH variations is crucial for the examination of diverse biological processes, such as cell cycle and apoptosis, cell proliferation as well as tumor growth. Among various approaches applied to examine the pH-induced fluctuations, optical methods show a significant advantage due to a low cost, rapid response, high sensitivity and non-invasiveness [2]. The examination of C-dots optical properties in a broad pH range revealed that both the PL intensity (*Fig. 2A*) and the average PL lifetimes (*Fig. 2B*) of C-dots dispersion are significantly diminished in an acidic environment, thus revealing that carbogenic nanoparticles can be utilized as biocompatible nanoprobes for an early-stage disease diagnosis.

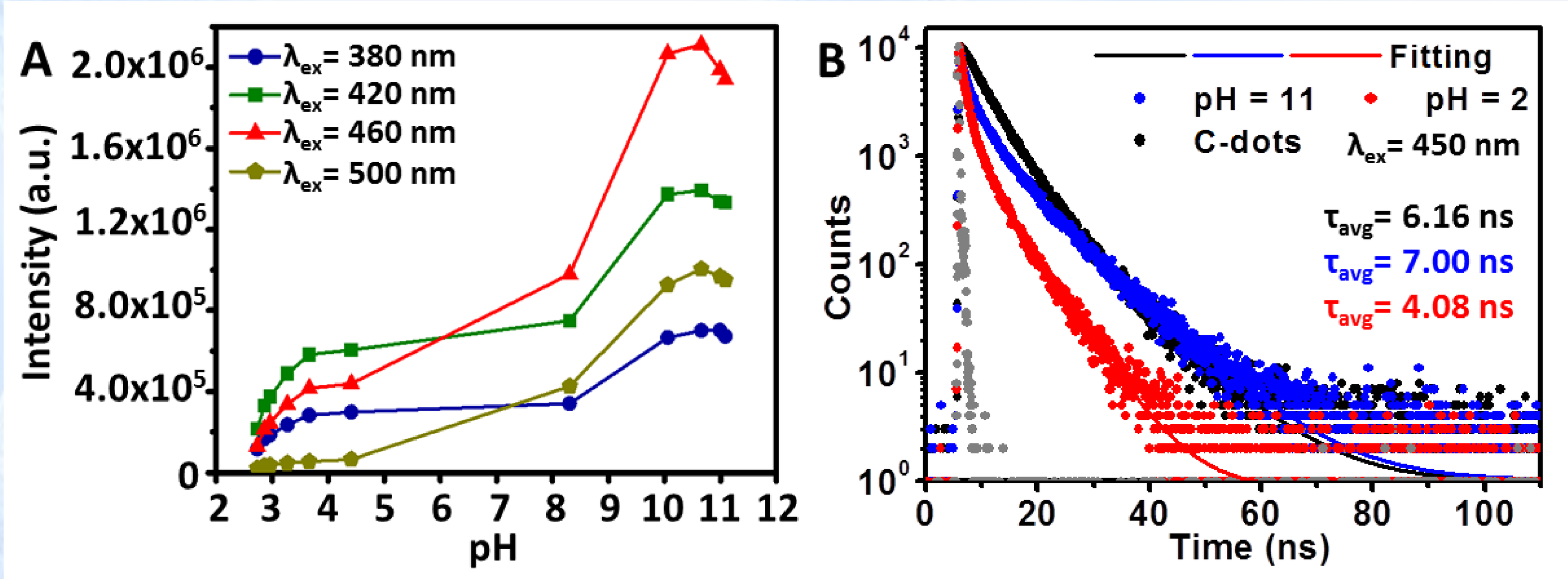


Figure 2. Plots presenting the pH dependence of PL intensity recorded at various excitation wavelengths (A) as well as PL lifetimes measured at λ_{ex}= 450 nm (B) for C-dots aqueous dispersion.

CYTOTOXICITY AND BIOIMAGING RESULTS

Prior to the utilization of synthesized carbogenic nanotracers for potential biomedical applications, the *in vitro* toxicity of C-dots (*Fig. 3A*) was evaluated against cancer HeLa cell line, with the use of MTT assay. The high viability of HeLa cells (>96%) after incubation with various concentrations of C-dots solutions (up to 400 μg/mL) for 24 and 48 hours confirmed their negligible cytotoxicity and suggested promising application in cellular imaging. Further investigation with the use of fluorescence microscope revealed the complete uptake of carbon dots by cancer cells, which were localized in both the cell membrane as well as perinuclear cytoplasmic region. The unchanged cells morphology under the bright field (*Fig. 3B*) confirmed their non-toxic nature, while the illumination of the cells with internalized C-dots by different excitation wavelengths displayed their multicolor nature, attributed to blue (*Fig. 3C*), green (*Fig. 3D)* and red *(Fig. 3E)* fluorescence.

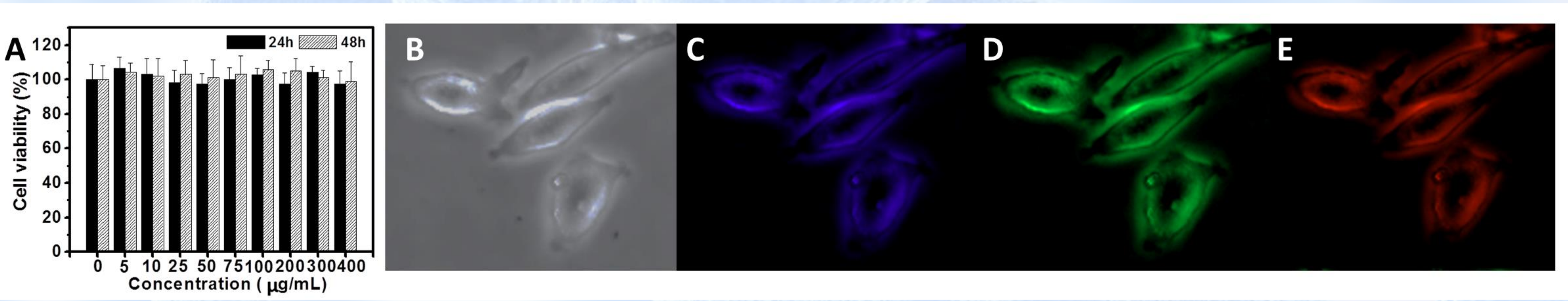
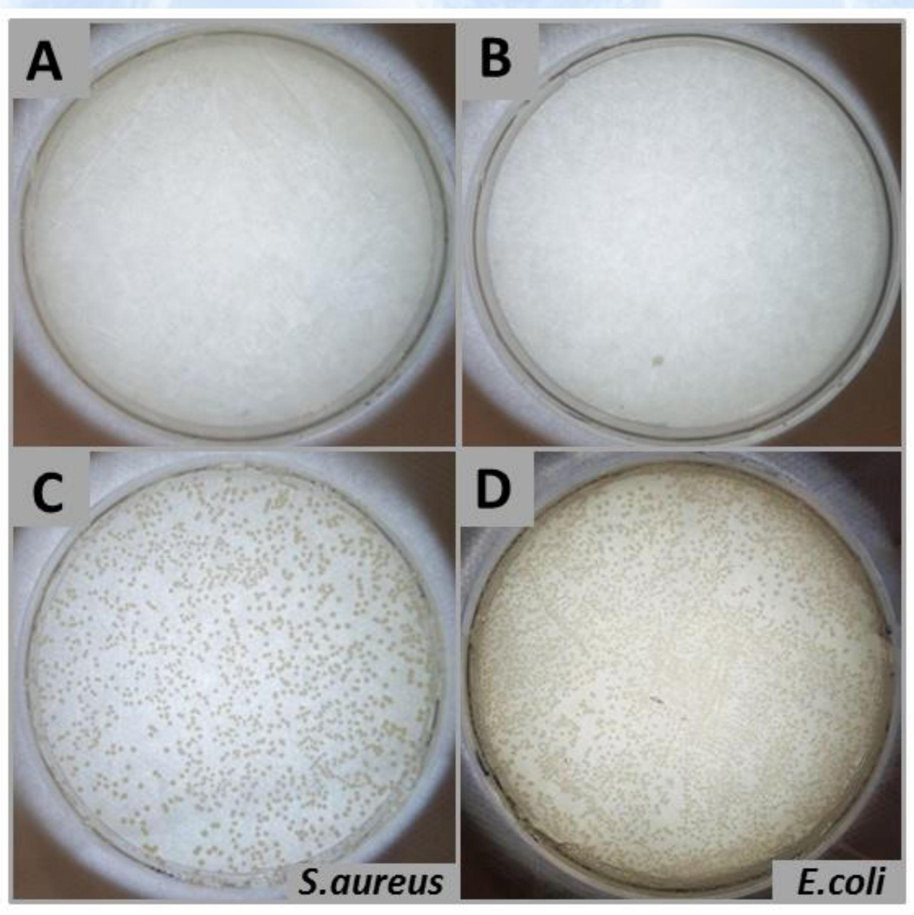


Figure 3. The MTT assay results presenting the cell viability of cancer HeLa cell line after incubation with various concentrations of carbogenic nanotracers for 24 and 48 hours at 37°C (A) together with the fluorescence microscope images of HeLa cells with internalized C-dots, under the bright field (B) as well as ultraviolet (C), blue (D) and green (E) excitation wavelengths (λ_{ex}= 366nm, 488nm, 566nm).

ANTIMICROBIAL PROPERTIES



The antimicrobial activity of C-dots against Gram-negative ***Escherichia coli*** as well as Gram-positive ***Staphylococcus aureus*** confirmed their excellent instant (>99.0%) as well as long-lasting (>99.8%) antimicrobial effect, evaluated with the use of previously reported testing method [4]. In addition, the analysis of agar plates with bacteria and C-dots revealed the reduction of bacterial colonies (*Fig. 4A,B*), in contrast to the control plates with ***S.aureus*** (*Fig. 4C*) or ***E.coli*** (*Fig. 4D*).

Figure 4. Photos of agar plates incubated for 2 hours (A) and 24 hours (B) at 37°C with bacteria colonies and C-dots together with control plates containing only S.aureus (C) or E.coli (D) cultures incubated for24 hours.

CONCLUSIONS

To date, various fluorescent sensors have been developed, including fluorescent proteins, organic dyes or quantum dots. Among them, C-dots display some beneficial features, such as good permeability, resistance to photobleaching and lack of toxic metal components in their structure. In addition, carbogenic nanoparticles demonstrate excellent analytical performance in detecting heavy metals, drugs, biological molecules, poisonous reactants or explosives, thereby can be utilized as a highly selective optical nanoprobes[2,5]. The nanoscopic size of carbogenic nanotracers enables their uptake by human or bacterial cells, while multicolor nature allows to illuminate the cellular components and gain complex information about various physiological and pathological molecular processes. On the other side, the remarkable antimicrobial properties of cabogenic nanotracers could help to overcome the spread of infectious bacterial diseases as well as limit the use of bacteria-resistant antibiotics.

BIBLIOGRAPHY

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