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In vitro antibacterial activity and biocompatibility of chiral lysine carbon dots

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Abstract: L-/D-lysine carbon dots (L-/D-Lys CDs) were synthesized and subsequently evaluated for their biocompatibility and in vitro antibacterial efficacy against both Gram-positive and Gram-negative bacteria. Chiral lysine carbon dots (L-Lys CDs and D-Lys CDs) were synthesized via a surface chiral modification strategy. Their chiral and spectral properties were characterized by transmission electron microscopy, circular dichroism spectroscopy, fluorescence spectroscopy, and X-ray photoelectron spectroscopy. The in vitro antibacterial activity of the two chiral carbon dots was assessed against four standard strains (*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*), two clinical isolates (*Acinetobacter baumannii*, *carbapenem-resistant Klebsiella pneumoniae*), and two drug-resistant strains (*carbapenem-resistant Acinetobacter baumannii*, *methicillin-resistant Staphylococcus aureus*). Their in vitro biocompatibility was also preliminarily investigated. The chiral lysine carbon dots preserved intact chirality, exhibited a relatively uniform particle size distribution (mostly 3-6 nm), and possessed good fluorescence properties. The minimum inhibitory concentration (MIC) values of L-Lys CDs against eight tested strains (four standard strains and four clinical isolates) ranged from 0.48 to 2.42 mg/mL, whereas those of D-Lys CDs ranged from 3.32 to 8.28 mg/mL. L-Lys CDs showed 2.8- to 15-fold lower MIC values compared with D-Lys CDs, demonstrating a marked stereoselective antibacterial advantage. Notably, the MIC of L-Lys CDs against carbapenem-resistant Acinetobacter baumannii was as low as 0.48 mg/mL, and that against methicillin-resistant Staphylococcus aureus was 0.96 mg/mL. Both MTT cytotoxicity assay and hemolysis test indicated that the chiral lysine carbon dots possessed good in vitro biosafety. L-Lys CDs exhibit favorable in vitro antibacterial activity against a variety of bacteria, and their in vitro biocompatibility meets the blood safety requirements of international standards for clinical drugs.

1. Introduction

Bacterial infection remains one of the major threats to global public health. Although the widespread use of antibiotics has significantly reduced mortality from infectious diseases, the

consequent problem of antimicrobial resistance (AMR) has become increasingly severe. The continuous emergence of drug-resistant strains such as *methicillin-resistant Staphylococcus aureus* (MRSA) and *carbapenem-resistant Acinetobacter baumannii* (CR-AB) has dramatically diminished the therapeutic efficacy of conventional antimicrobial agents [1,2]. Therefore, developing novel antibacterial materials with new mechanisms of action, low propensity for inducing resistance, and good biocompatibility is of great clinical importance.

Carbon dots (CDs) are a class of quantum-sized fluorescent nanoparticles typically less than 10 nm in diameter. They possess advantages such as facile preparation, excellent water solubility, easy surface functionalization, and low toxicity, while also exhibiting outstanding photoluminescence properties. Consequently, CDs have been widely applied in various biomedical fields, including live-cell imaging, catalysis, electronics, biosensing, targeted drug delivery, and other biomedical applications [3–8]. In recent years, researchers have found that surface modification with amino acids, quaternary ammonium salts, and other groups can significantly enhance the antibacterial activity of CDs [9,10]. However, existing CD-based antibacterial strategies mostly rely on physical membrane disruption or photocatalytically generated reactive oxygen species (ROS), which have limitations such as single mechanism of action and potential damage to normal cells [11].

Chirality, a fundamental feature of living systems, plays a key role in molecular recognition and biological metabolism. Studies have shown that bacteria exhibit a natural metabolic preference for L-amino acids, and specific L-amino acid transport systems exist on their cell membranes [12,13]. Based on this, using chiral ligands to modify CDs offers a promising “metabolic interference” strategy: by mimicking essential bacterial nutrients, these chiral CDs can be deceptively internalized into the bacterial cytoplasm, where they disrupt key metabolic pathways, thereby achieving high antibacterial efficacy with low toxicity and low risk of resistance development [14,15].

Lysine is an essential basic amino acid, and its L- and D-enantiomers differ significantly in structure and biological activity. Currently, a systematic evaluation of the differences in in vitro antibacterial activity and biocompatibility between chiral lysine carbon dots derived from L- and D-lysine is still lacking. In this study, citric acid was used as the carbon source, and L-lysine and D-lysine were employed as chiral modification agents to prepare two types of chiral lysine carbon dots (L-lys CDs and D-lys CDs). After characterizing their physicochemical properties, we systematically compared their in vitro antibacterial activities against standard strains and clinical drug-resistant isolates, and evaluated their cytotoxicity and hemocompatibility. Our aim is to provide experimental evidence for the development of chiral carbon dots as new topical antimicrobial materials.

2. Materials

2.1. Experimental strain

The E. coli Dh5a, K. pneumoniae NTUH-K2044, P. aeruginosa PAO1, S. aureus SA1-SA29, and ATCC 43300 strains were kindly provided by the Department of Health Laboratory Technology, College of Public Health, Chongqing Medical University. The clinical isolates K. pneumoniae (Kpn), A. baumannii (Aba), and carbapenem-resistant A. baumannii (CR-AB) were obtained from sputum samples of patients at the University Town Hospital affiliated with Chongqing Medical University.

2.2. Experimental cell

The A549, MCF-7, HEK293T, MDA-MB-231, and L02 cell lines were kindly provided by the Department of Clinical Diagnostics, College of Laboratory Medicine, Chongqing Medical

University.

2.3. Main instruments and reagents

Anhydrous citric acid, L-lysine, D-lysine, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were purchased from Titan (Shanghai, China). Tryptone, yeast extract, and agar were obtained from Thermo Fisher Scientific (USA) and Beijing BRJ (Beijing, China), respectively. Glucose monohydrate was from Aladdin (Shanghai, China). DMEM medium, fetal bovine serum (FBS), penicillin-streptomycin (100×), and 0.25% trypsin-EDTA solution were supplied by Beyotime (Shanghai, China).

The following instruments were used for characterization: a transmission electron microscope (JEM-F200, JEOL, Japan) and a circular dichroism spectrometer (J-1500, JASCO, Japan).

3. Methods

3.1. Preparation of chiral lysine carbon points

First, 20 g of anhydrous citric acid was dissolved in 200 mL of ultrapure water, ultrasonicated for 5 min, and then hydrothermally treated at 180 °C for 4 h to obtain CA-CDs. Subsequently, 80 mL of the CA-CDs solution was mixed with 0.5 g of L- or D-lysine and stirred vigorously at 1500 rpm for 24 h at room temperature to produce the crude L-/D-Lys CDs solution.

Each crude solution was centrifuged at 12,000 rpm for 10 min and filtered through a 0.22 μm cellulose acetate membrane to remove large particles. The supernatant was then dialyzed against deionized water using a 1000 Da dialysis bag for 24 h, with the dialysis medium changed every 3 h to eliminate residual small-molecule impurities. Finally, the dialysate was concentrated at 60 °C and freeze-dried to obtain a light-yellow powder, which was stored in sealed containers at room temperature for subsequent use.

3.2. Characterization of carbon dot of chiral lysine

The morphology of the as-prepared carbon dots were studied by transmission electronic microscopy (JEOL JEM-F200,Japan).The chiral properties of carbon dots are measured by circular dichroism (CD) spectroscopy (JASCO J-1500,Japan).

3.3. Detection of antibacterial effect in vitro

Ec. and SA. strains were inoculated into LB broth and incubated overnight at 37 ℃ with shaking at 200 rpm. Then the bacterial suspension was diluted with LB broth, so that the OD of the bacterial suspension was 0.1 (1×10^8 CFU/mL) and the wavelength was 600. Then the bacterial suspension was diluted 1: 200 (5×10^5 CFU/mL) with LB + 0.5% glucose medium for later use.

Add 100 μL, 90 μL, 80 μL, 70 μL, 60 μL, 50 μL, 40 μL, 30 μL, 20 μL, 10 μL, 0 L-lysine carbon dots into the three parallel rows of A/B/C holes of a 96-well plate according to different volume ratios, and then add 0, 10 μL and 20 μL into the three parallel rows of A/B/C holes respectively. Through three parallel experiments, 50 μL of diluted bacteria solution was added to each well of the three rows of A/B/C, making the total reaction system 150 μL. In row D, 150 μL of LB + 0.5% glucose medium was set as a blank control, and in row E, 150 μL of diluted bacterial solution was set as a negative control.

The gun was transferred to the LB plate, then it was evenly coated with a coating stick, and it was cultured at 37℃ for another 24 hours. Shake the mixed solution of the 96-well plate with an

oscillator, put it in a constant-temperature incubator, and cultivate it at 37℃ for 18—24 hours. After the culture, the liquid in the aseptically grown holes and the holes with the above concentration was transferred to the LB plate with a pipette, and then evenly coated with a coating stick, and then cultured at 37℃ for another 24 hours.

Results Interpretation: The MIC of chiral lysine carbon point to bacteria is the lowest concentration that the chiral lysine carbon point drug can completely inhibit the growth of bacteria with naked eyes, that is, the culture medium in the hole is completely clear and there is no bacterial growth. The minimum carbon point concentration of chiral lysine for aseptic growth on LB plate is MBC, that is, the minimum bactericidal concentration.

3.4. In vitro cytotoxic effect

L02 cells in the logarithmic growth phase were seeded into 96-well plates at a density of 0.5×10^4 cells per well, with 100 μL of complete culture medium added to each well. The plates were incubated at 37 °C in a 5 % CO_2 atmosphere for 12 h to allow cell attachment. Five concentration gradients of L-/D-lysine carbon dots (5, 50, 500, and 5×10^3 μg/mL) were set as treatment groups, each with three replicate wells. A negative control group (cells without carbon dots) and a blank control group (culture medium without cells or carbon dots) were also included.

The old culture medium was aspirated, and fresh complete medium containing the respective concentrations of carbon dots was added. After further incubation for 24 h, 20 μL of MTT solution (5 mg/mL) was added to each well, and the plates were incubated for an additional 4 h at 37 °C. The supernatant was carefully discarded, and 150 μL of dimethyl sulfoxide (DMSO) was added to each well. The plates were shaken on a microplate shaker for 10 min to completely dissolve the purple formazan crystals. Finally, the absorbance of each well was measured at 490 nm using a microplate reader (OD_{490}). Cell viability was calculated using the following formula:

$$\text{Cell viability (\%)} = [(A_s - A_b) / (A_c - A_b)] \times 100\ \% \quad (1)$$

Where A_s is the absorbance of the experimental group, A_b is the absorbance of the blank control group, and A_c is the absorbance of the negative control group.

3.5. Hemolysis assay

Fresh blood was taken to an anticoagulant tube, transferred to a centrifuge tube, and centrifuged at 1500 rpm at 4℃ for 5 minutes, so that red blood cells were precipitated to the bottom of the tube, the supernatant was discarded, and a proper amount of normal saline was added to gently suspend red blood cells, and then centrifuged again to remove impurities and substances that might affect the experiment. Add 1 mL of three carbon point solutions, namely CA-CDs, L-lys CDs and D-lys CDs, to red blood cells, incubate at 37℃ for 0.5 hour, collect the supernatant by centrifugation and transfer it to a 96-well plate. Deionized water and 0.9% saline solution were added as negative control and positive control, respectively. The absorbance of the supernatant at 540nm was measured, and the hemolysis rate was calculated according to the following formula [16]:

$$\text{Hemolysrate (\%)} = [(A_1 - A_0)/(A_2 - A_0)\ ] \times 100\% \quad (2)$$

Where A_1 is the absorbance (OD_{540}) of three carbon point solutions, A_0 is the absorbance of physiological saline (OD_{540}) and A_2 is the absorbance of deionized water (OD_{540}).

3.6. Statistical analysis

All experiments were performed in triplicate, and the data are presented as mean ± standard

deviation (SD). Statistical analyses were conducted using SPSS software (version 26.0). One-way analysis of variance (ANOVA) was used for between-group comparisons, followed by Duncan's post-hoc test for multiple comparisons. The significance level was set at $\alpha = 0.05$, and a P-value of less than 0.05 was considered statistically significant.

4. Results

4.1. Characterization of chiral lysine carbon dots

TEM characterization (Fig. 1) revealed that L-lys CDs had an average diameter of 4.67 ± 0.86 nm, and high-resolution TEM images showed clear lattice fringes on the carbon dot surfaces with a spacing of 0.357 nm. D-lys CDs exhibited an average diameter of 4.77 ± 0.33 nm and a lattice spacing of 0.352 nm. Circular dichroism (CD) spectra demonstrated that L-lys CDs and D-lys CDs displayed distinct, mirror-symmetric positive and negative signal peaks at a wavelength of 201 nm, indicating that their chiral structures remained intact.

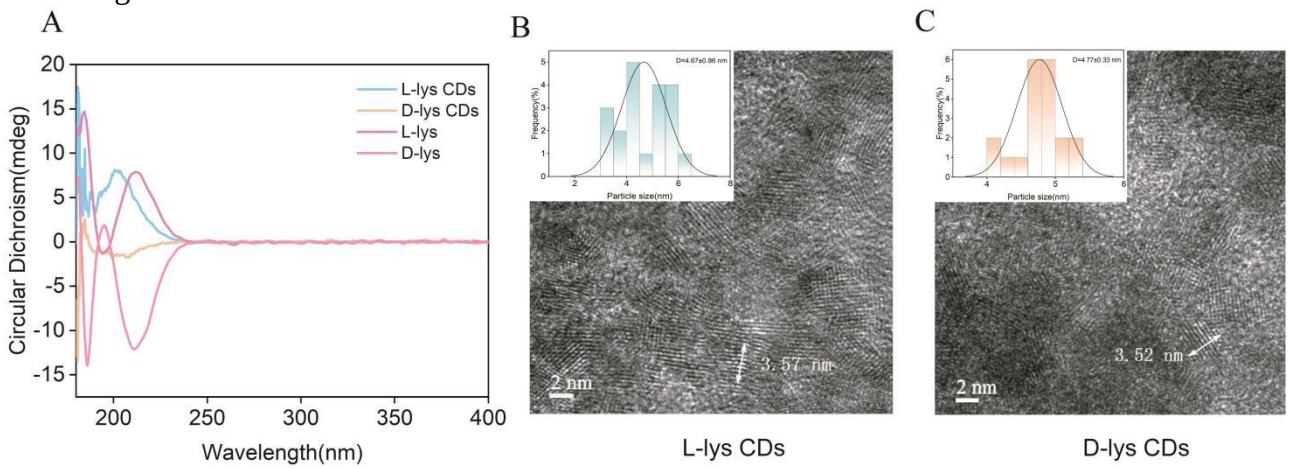

Figure 1: Chiral Properties of L-lys CDs and D-lys CDs.

4.2. In vitro antibacterial activity of chiral lysine carbon dots

L-lys CDs exhibited significantly stronger antibacterial activity than D-lys CDs and CA-CDs. The minimum inhibitory concentration (MIC) values of L-lys CDs against the eight tested bacterial strains ranged from 0.48 to 2.42 mg/mL, whereas those of D-lys CDs ranged from 3.32 to 8.28 mg/mL. Compared with D-lys CDs, L-lys CDs showed a 2.8- to 15-fold reduction in MIC, indicating a marked stereoselective antibacterial advantage (Fig. 2).

Notably, L-lys CDs displayed potent activity against clinically relevant drug-resistant strains: the MIC against *carbapenem-resistant Acinetobacter baumannii* (CR-AB) was as low as 0.48 mg/mL, and the MIC against *methicillin-resistant Staphylococcus aureus* (MRSA) was 0.96 mg/mL. Furthermore, the MIC of L-lys CDs against both *Escherichia coli* and *Staphylococcus aureus* was 0.5378 mg/mL, while the MIC values against the clinical isolates *Acinetobacter baumannii* and *carbapenem-resistant Klebsiella pneumoniae* were 2.416 mg/mL and 2.24 mg/mL, respectively.

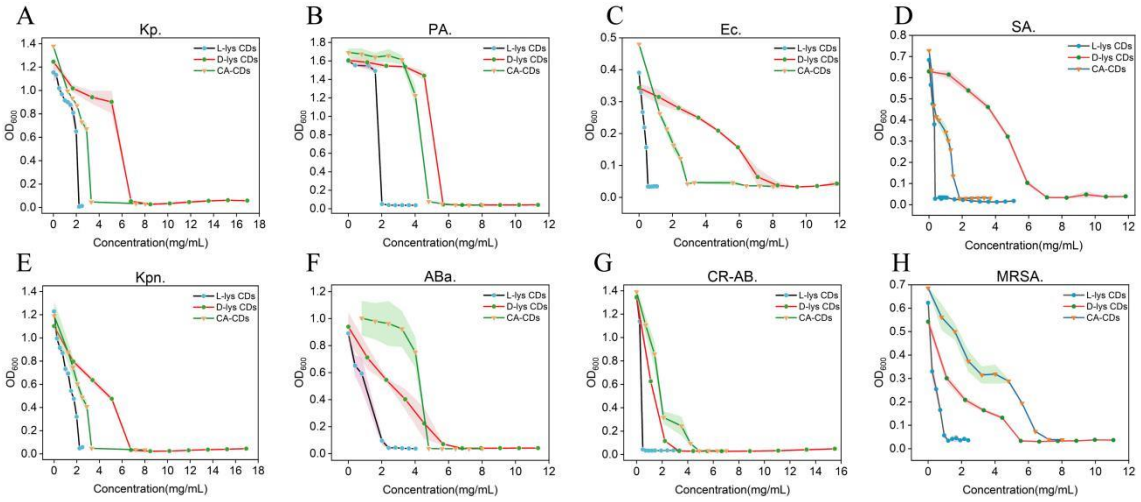

Figure 2: Antimicrobial activity of L-lys CDs, D-lys CDs and CA-CDs at different concentrations on eight kinds of bacteria.

4.3. Biocompatibility

MTT assays showed that L-/D-lys CDs at up to 5000 μg/mL resulted in >80% cell viability in all five cell lines. Hemolysis rates for CA-CDs, L-lys CDs, and D-lys CDs were 0.59%, 1.22%, and 3.20%, respectively, all <5% (Fig. 3) .

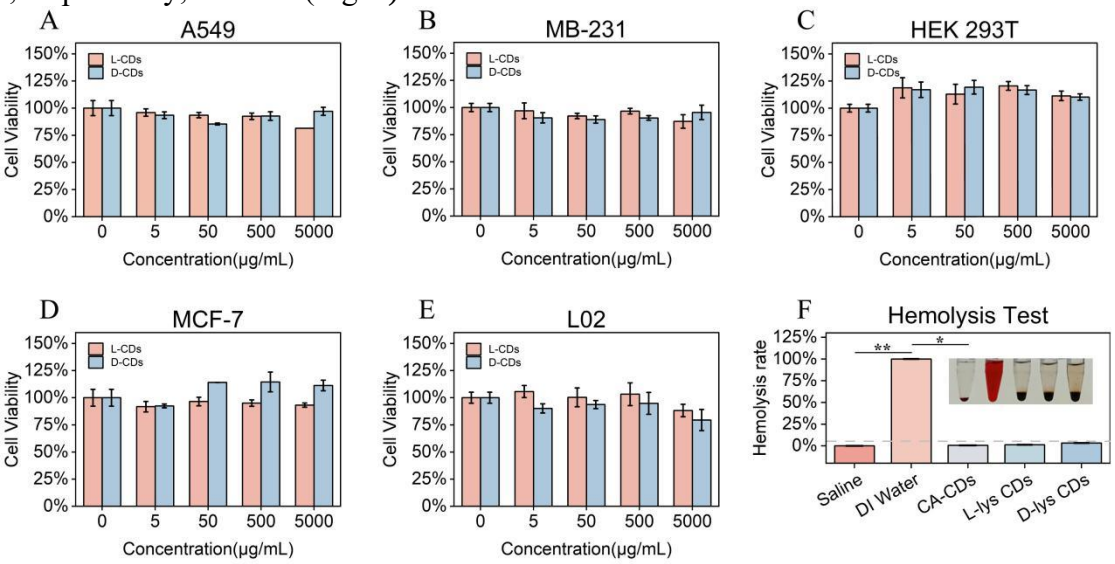

Figure 3: Biosafety evaluation of L-lys CDs and D-lys CDs.

5. Conclusions

In this study, chiral lysine carbon dots (L-Lys CDs and D-Lys CDs) were successfully synthesized using a surface chiral modification strategy. L-Lys CDs exhibited markedly higher stereoselective antibacterial activity than D-Lys CDs against a broad spectrum of bacteria, including standard strains and clinically isolated drug-resistant pathogens such as CR-AB and MRSA. The minimum inhibitory concentrations of L-Lys CDs were 2.8- to 15-fold lower than those of D-Lys CDs, demonstrating a clear chirality-dependent advantage. Furthermore, MTT cytotoxicity and

hemolysis assays confirmed that L-Lys CDs possess good in vitro biocompatibility, with cell viability exceeding 80% at concentrations up to 5000 μg/mL and a hemolysis rate of only 1.22%, well below the 5% safety threshold for clinical use. These findings indicate that L-Lys CDs are promising candidates for the development of novel topical antimicrobial agents with low toxicity and a low propensity for inducing drug resistance. Further in vivo studies and mechanistic investigations are warranted to fully explore their therapeutic potential against drug-resistant bacterial infections.

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