Synthesis of carbon dots and their application in biomedical research

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Abstract. Carbon dots (CDs) are novel carbon-based zero-dimensional nanomaterials. compared with conventional semiconductor quantum dots, carbon dots have the advantages of stable fluorescence characteristics, good biocompatibility, low toxicity and easy functionalization improvement. Based on this, the synthesis methods of carbon dots and new strategies in synthesis are introduced. then, the excellent fluorescence properties and unique antibacterial efficacy of carbon dots were studied. Combined with the latest clinical medical advances, the potential value of carbon dots in bioimaging, guiding tumor metastasis, postoperative anti-inflammatory and other fields was summarized.

1. Introduction
Carbon is the most basic element of organic matter, which exists in a wide range of forms. Among them, Carbon dots (CDs) are nano-materials with superior optical properties discovered in recent years, which are generally less than 10 nm in size and include amorphous or crystalline sp² hybrid carbon[1]. Compared with traditional organic dyes and semiconductor quantum dots, CDs has the characteristics of wide raw material sources, simple synthesis method, wide application range, strong optical stability, low toxicity and good biocompatibility. The luminescent properties of nano-CDs can be divided into photoluminescence and electrochemical luminescence, among which fluorescence and phosphorescence belong to luminescence and are widely used. Recently, it has been reported that CDs modified by -OH/-COOH, -NH, PEG has very low toxicity to mouse cells[2]. After human neural stem cells absorb CDs, their cell activity, reproduction, metabolic activity and disproportionation potential have no obvious changes[3]. The very stable fluorescence characteristics (PL) and extremely low cytotoxicity of CDs are good candidates for real-time study of living cell biological imaging of dynamic cell processes. In recent years, the synthesis of near infrared (NIR) absorption/emission CDs has made great progress in the field of nanomedicine. Biomolecules are transparent to near infrared radiation, so they can be used as in vivo imaging probes. In the near future, phosphorescence will change the field of biological imaging, mainly because phosphorescence life is longer than fluorescence, so more complex phosphorescence medical tools can see the complete dynamic process in cells[4]. CDs also has important application value in testing and treating cancer and biological antibacterial property in biomedical field. Heavy metal semiconductor quantum dots are very toxic in medical applications. Even if the concentration of heavy metals is very low, they will react with the body and cause harm. For example, carbonyl and amine modified quantum dots will cause blood coagulation and blood vessel thrombosis[5], which makes it inevitable that CDs with the properties of metal-like quantum and low toxicity will replace metal quantum dots. Nowadays, CDs has been
widely used in the field of medicine, and it has wide application value in the fields of biomarkers, biosensing, biological imaging, biochemical analysis, photocatalysis and drug carriers.

2. Synthesis methods of CDs

2.1. Current strategy of CDs synthesis
At present, the synthesis methods of CDs can be divided into two categories: "top-down" and "bottom-up" methods. "Top-down" is to split the large-size carbon source precursor into CDs by physical and chemical methods, such as peeling off the electrochemical skin, oxidizing acid treatment, laser ablation, arc discharge, etc. under relatively severe heating conditions. Most of the CDs prepared are less than 10 nm in diameter\[6\]. Electrochemical synthesis is the preferred method to synthesize homogeneous CDs. The uniformity of topography is usually achieved by changing the applied potential on the electrode. However, the strong acid used in the top-down synthesis method and the complicated cleaning and dialysis process, coupled with the need to adjust PH, do great harm to biological cells. This method is not conducive to the preparation of CDs for biological applications. In the "top-down" approach, a single step is required to synthesize CDs passivation, but in the "bottom-up" approach, no single step is required, mainly because surface passivation can be done in one pot. In addition, small organic precursors can be polymerized and pyrolysis carbonized into CDs, microwave polymerized and carbonized \[7\]. Next, I mainly talk about the following preparation methods, and put forward their latest progress.

2.2. Hydrothermal synthesis
Hydrothermal/solvothermal method is an economical, green and easy-to-operate method, which can synthesize CDs from various carbon-based precursors. Hydrothermal synthesis is to use a high temperature and high pressure environment to break the original carbon structure into small molecular carbon, and make it self-polymerize, forming carbon nucleus and then polymerizing into CDs. For example, the epoxy groups on the graphite surface are arranged linearly, which are fragile and easily break into small molecular carbon at high temperature, and finally form carbon spots\[8\]. The fluorescence intensity (PL) of CDs can be adjusted by changing the molar mass of precursor, physicochemical properties of solvent, heating time and temperature. For example, Sudolska et al. prepared full-color CDs by hydrothermal method with citric acid and urea as raw materials and N, N-dimethylformamide as environment, and controlled the fluorescence at blue, green, yellow or red wavelengths\[9\]. The above synthesis strategy indicates that fluorescent CDs can be easily prepared in large quantities and has important biomedical applications.

2.3. Microwave assisted thermal synthesis
Microwave assisted heating is a development of hydrothermal technology which uses microwave instead of heat. Carbon-based materials have strong interaction with microwave, which is the key factor to realize efficient local heating. Controlling microwave power is simpler than controlling water temperature, and it is easy to heat locally at high speed, which is conducive to accelerating the carbonization process and contributing to the emergence of unique morphology of nanostructures. Microwave method can synthesize a series of different nano CDs by controlling microwave power, reaction time and pH value\[10\].Teves' team\[11\] reported an efficient and controllable method for synthesizing CDs by microwave heating the mixture of citric acid and branched polyethyleneimine. this method changed the internal structure of CDs and increased the versatility and luminescence specificity of CDs. moreover, CDs synthesized by this method can be modified between luminescence and catalytic performance by simple synthesis operation. Recently, Jiang et al\[12\] proposed a fast and simple method for synthesizing ultra-long room temperature phosphorescence (URTP) CDs by microwave heating. The method is prepared by microwave-assisted carbonization of ethanolamine and phosphoric acid, and the phosphorescence life of the prepared CDs is very long, which is 1.46s (about 10s with naked eyes), As shown in fig. 1.
2.4. Ultrasonic synthesis

Ultrasonic synthesis does not need a large amount of solvent, and the reaction time is very short, but the reaction is not easy to control and side reactions occur. Ultrasonic wave is used to generate pressure difference and shear force in graphite to break chemical bonds and make in situ carbon passivate to form carbon points. A large number of carbon points were synthesized by this method, and most of them emitted blue fluorescence\cite{13}. However, the wavelength of blue-purple fluorescence was too short to dialysis the skin, making it difficult to conduct biological imaging. Liu\cite{14} et al. used candle ash as carbon source to synthesize yellow-green fluorescent CDs with uniform particle size by ultrasonic method in strong acid environment. The synthesized carbon dots have yellow-green fluorescence, long wavelength, good photobleaching resistance and biocompatibility, and can potentially be used as yellow-green fluorescent imaging reagents for cell imaging.

3. Biomedical applications

3.1. Bioimaging

CDs has similar fluorescence characteristics to heavy metal clusters, but its cytotoxicity is extremely low, which makes them powerful candidates for designing new biological imaging probes. However, most CDs show short-wavelength fluorescence, which will produce autofluorescence and interfere with the imaging effect when penetrating biological tissues. However, long-wavelength CDs can easily penetrate deep tissues of the body and facilitate biological imaging. Teng et al.\cite{15} synthesized nitrogen-doped N-CDS and cultured human cervical cancer cells in different channels for 2 h, and the fluorescence imaging of HeLa cells was clearly visible. In contrast, HeLa cells untreated with N-CDS did not show any fluorescence. Wang's team synthesized a new type of CDs (S, N-CDS) doped with ammonia and sulfur, and carried out cell uptake experiments in MCF7 in vitro, which were recorded by laser scanning confocal microscopy (LSCM). The bright field image of MCF7 showed normal morphology, indicating that S, N-CDS had good biocompatibility and minimal cytotoxicity. The cells incubated with S and N-CDS can simultaneously detect the cell membrane and cytoplasm of McF-7, which can be used as an ideal probe to detect HOCl in organisms\cite{16}.

3.2. Future application direction of CDs phosphorescence

At present, the research of phosphorescent materials is still limited to metal-organic composite materials. Compared with fluorescence, phosphorescence has a longer life, and can completely present the dynamic process of cells. The phosphorescence phenomenon of carbon dots was originally described by Deng et al.\cite{17}. As shown in fig.2, when the carbon dots with blue fluorescence are irradiated by the excitation light source to form a block with polyvinyl alcohol (PVA), fluorescence can be observed, and when the excitation light source is turned off, green phosphorescence visible to the naked eye can be observed, but the same carbon dots have no phosphorescence phenomenon in water, because phosphorescence wavelength will be converted into fluorescence wavelength in water, so PVA matrix has an important protective effect on the formation of triplet with long-life emission. Among them, hydrogen bonds can fix luminescent groups and effectively prevent intramolecular movement, thus reducing non-radiative transition and enhancing phosphorescence. If the problem of
phosphorescence disappearing in water can be solved, phosphorescence can be applied to clinical medicine.

![Coated FNCDs on paper](image)

**Fig. 2** The fluorescence of CDs disappears and phosphorescence appears\[^{17}\]

### 3.3. **Antibacterial activity and sterilization mechanism of R-CDs**

In recent years, multicolor luminescent CDs has attracted people's attention because of its wide application. Especially some rare red fluorescent CDs (R-CDs), most of which are made of toxic raw materials, but there are also some natural materials, such as melanin and betaine hydrochloride. The molecular structure of betaine hydrochloride is mainly quaternary ammonium cation, which makes the surface of prepared R-CD contain quaternary ammonium cation, then the surface of the synthesized R-CDs has abundant positive charges, and the quaternary ammonium cation has effective bactericidal activity\[^{18-19}\]. When R-CDs is with bacteria, it can be combined with negatively charged bacteria by electrostatic action, which makes quaternary ammonium cation contact with bacterial membrane, changes the permeability of membrane, and then causes lysis, destroys cell structure and causes cell dissolution and death, as shown in fig.3. According to research, the effect of R-CDs on Gram-negative bacteria is more significant than that on Gram-positive bacteria, especially on Escherichia coli.

![Mechanism of CDs destroying bacterial structure and causing bacterial extinction](image)

**Fig.3** Mechanism of CDs destroying bacterial structure and causing bacterial extinction\[^{18}\]

### 4. **Conclusion**

At present, CDs has been widely used in the fields of biological imaging, fluorescent materials and biosensing. In particular, phosphorescence has a longer life than fluorescence, and can be applied to long-term biological imaging, so that different cell dynamic processes can be understood more deeply. The unique PL properties, strong photodynamic activity, good biocompatibility and stable antibacterial activity of CDs materials make CDs materials have many potential application values in biomedicine. Such as early diagnosis of cancer, dynamic-real-time location agent in vivo, image guidance and treatment of tumor metastasis, postoperative anti-inflammation, and so on. To sum up, CDs has a lot of research value and broad application prospects in the fields of nanomedicine and nanosurgery, and it is expected that the clinical application of CDs will become a reality in the near future.

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