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Carbon dots in biomedicine: From synthesis to application

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Abstract. Many studies have examined the properties and possible uses of carbon dots (CDs), a novel class of zero-dimensional carbon nanomaterials. Many notable features are present in them, such as robust colloidal stability, easy surface functionalization and bioconjugation, robust biocompatibility in vivo and in vitro settings, resistance to photobleaching, an environmentally benign synthesis process, and a comparatively low production cost. These characteristics make carbon dots an attractive option for a variety of possible biomedical uses. This work provides a thorough review of the most recent developments in the biomedical sciences, covering the physicochemical characteristics, synthesis techniques, and uses of carbon dots in biosensing, bioimaging, and disease treatment. It is based on a thorough examination of the relevant literature and experimental results. According to the study findings that are now accessible, carbon dots have a lot of potential applications in the biomedical industry. These properties make carbon dots suitable for use in bioimaging, biosensing, and disease treatment. Nevertheless, there are still some challenging issues that require further investigation and analysis. It is anticipated that further research and exploration will result in carbon dots making a significant contribution to human health in the future, and that the ongoing innovation and development of carbon dot technology will result in a significant transformation of the biomedical field.

Keywords: Carbon Dots, Synthesis, Biosensing, Bioimaging, Treatment of Disease.

1. Introduction

Recently, there have been many opportunities for the use of carbon dots (CDs), a zero-dimensional carbon nanomaterial with special optical and chemical properties, in the biomedical industry. Carbon dots have attracted great attention from researchers around the world because of their low cytotoxicity, excellent biocompatibility, and tunability. The first unintentional discovery of CDs occurred in 2004 while purifying single-walled carbon nanotubes made using the arc discharge technique; subsequently, Sun et al. named this new material in 2006 [1]. Carbon dots are mainly divided into three types: carbon quantum dots (CQDs), graphene quantum dots (GQDs) and carbonized polymer dots (CPDs) [2]. The remarkable optical, electrical, and chemical properties of carbon dots, which have a size range of a few nanometers up to tens of nanometers, make them perfect for a range of biomedical applications, such as biosensing, bioimaging, and disease treatment. In this paper, recent advances in synthesizing carbon dots for biomedical applications are discussed, with emphasis given to the synthesis, characterization, and application of carbon dots in bioimaging, biosensing, and disease treatment. In addition, challenges and future prospects for the development of carbon dots are discussed. Exploring the production of

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carbon dots and a variety of uses for them in the biomedical industry, this paper aims to provide insights into the use of these nanomaterials in biomedical diagnostics, therapeutics and imaging technologies.

2. Physicochemical Properties and Synthesis Methods of Carbon Dots

2.1. Physicochemical Properties

CDs often exhibit substantial UV absorption in optical absorption, with the major absorption peak being at approximately 250 nm, linked to the Π-Π* transition of the C=C bond. Additionally, some CDs exhibit distinctive absorption peaks in the visible-near infrared spectrum. Although the structure, size, and surface functional groups of CDs vary, all classes exhibit similar photoluminescence (PL) properties. CDs materials exhibit stable, easily tunable, and multi-color fluorescence properties. Because of the quantum size effect, the size of GQDs and CQDs and their emission wavelength are tightly correlated. Surface passivation or functionalization can also significantly affect PL properties. For example, polymer passivation can enhance fluorescence. Compared with CQDs and GQDs, the PL characteristics of CPDs are slightly different and generally have wider emission peaks and excitation dependence. By optimizing precursors and reaction conditions, CPDs with wide full width at half maximum (FWHM) and long-wavelength emission can be developed. Some CPDs also have long-term continuous luminescence properties, such as thermally activated delayed fluorescence and phosphorescence. CDs also exhibit up conversion photoluminescence (UCPL) properties, which can produce visible light emission under near-infrared excitation, which has potential applications in photocatalysis and bioimaging. Photoinduced charge migration is another important property of CDs.The conjugated carbon skeleton of CDs ensures good electrical conductivity and can serve as an electron acceptor or donor to participate in the charge migration process, which has advantages in the fields of photocatalysis and optoelectronics. Previous studies have confirmed that CDs can undergo processes such as charge separation, migration and recombination under light excitation by constructing a conjugated system of CDs and electron acceptors/donors.

2.2. Synthesis Method

Carbon dots can be made in a number of useful and efficient methods. In recent years, scientists have been studying how to synthesize carbon dots. The synthesis tactics of carbon dots can be broadly classified into two categories based on various carbon sources: "bottom-up" and "top-down" strategies [1]. Despite the variety of methods for synthesizing optical discs, there are still some problems, such as low efficiency, complex equipment and strict operational requirements. In the future, there is a need to continuously improve these methods to increase the quality and productivity of optical disks to meet the needs of a wider range of applications in the future. It is anticipated that the development of more effective synthesis techniques, including as microfluidics, ultrasound, and electromagnetic induction heating, may hasten the process of transferring optical disks from laboratory production to industrial use. The ensuing sections provide a detailed description of the "top-down" and "bottom-up" techniques.

2.2.1. The "Top-down" Strategy

Implementation of this approach necessitates the fragmentation of carbon allotropes, which occurs when larger carbon sources are physically or chemically separated into smaller carbon dots. Carbon nanotubes, carbon fibers, and graphite rods are typically used as carbon sources for "top-down" techniques. Arc discharge, laser ablation, oxidation, pyrolysis, ultrasonic, and ball milling are the primary techniques in this category. Among them, the arc discharge uses carbon soot in a low-voltage arc chamber to extract single-walled carbon nanotubes (SWNTs) and further purifies them to obtain CDs. Nevertheless, this process is not appropriate for large-scale manufacturing because to its uneven particle size distribution and low production efficiency. Laser ablation method quickly prepares CDs by irradiating solid carbon sources with high-energy laser, but the equipment is complex and there are safety risks. The oxidation method and the pyrolysis are relatively simple to operate, but the oxidation method requires special equipment or a large amount of reagents, and the pyrolysis method has strict requirements on reaction temperature and time. The ultrasonic method and the ball milling use ultrasonic waves and mechanical energy to prepare CDs respectively, and are low-cost, but the ball milling does not require harsh reagents and is environmentally friendly and simple.

2.2.2. The "Bottom-up" Strategy

Small carbon sources in ionic or molecular forms are used in this method. The most prevalent oligomers, or tiny organic compounds, include urea, glucose, citric acid, and polyethylene glycol. Bottom-up methods mainly include hydrothermal/solvothermal synthesis, microwave synthesis, template method, aldol condensation polymerization, electromagnetic induction heating methods, plasma method and microfluidic method. Hydrothermal/solvothermal synthesis and microwave synthesis are simple and efficient to operate and suitable for large-scale production. The template method can regulate the size and properties of CDs, but the process is complicated. The aldol condensation polymerization has mild reaction conditions, high yield and simple post-processing. The electromagnetic induction heating method has high heating efficiency and short reaction time. Plasma method and microfluidic method have the characteristics of efficient and precise synthesis, but they have high equipment requirements and high technical thresholds.

3. Carbon Dots in Biomedical Applications

3.1. CDs for Bioimaging

As nanomaterials with excellent fluorescence properties, high quantum yield, and outstanding biocompatibility, CDs have become ideal bioimaging probes and have shown unique attraction in bioimaging. The fluorescent features of CDs have been used in many investigations for both in vitro and in vivo biological imaging, including liver, lung, and other cancer cells.

Taking advantage of the optical properties of CDs, Liu et al. used a solvothermal synthesis to synthesize a red fluorescent carbon dot SOD nanozyme [3]. Superoxide dismutase (SOD) activity is connected to carboxyl, hydroxyl, and amino groups, as demonstrated by the passivation of functional groups. By forming hydrogen bonds with superoxide radicals, these surface functional groups quicken the radicals' breakdown. The CDs can remain inside the cells without being eliminated for up to 24 hours after they have entered there. This suggests that CDs can live steadily in the intracellular environment and have a lot of potential for bioimaging in vivo and in vitro as well as for treating disorders linked to ROS. In another study, Chen et al. used hydrothermal synthesis to create a type of S, N co-doped CDs with a high quantum yield and strong fluorescence stability [4]. They did this by employing L-cysteine as a precursor. Because S, N co-doped CDs have good biocompatibility and specific targeting ability, they can be used as fluorescent probes for in vitro fluorescence imaging of different cancer cells (breast cancer, melanoma, HeLa and glioma cells), and show good visual tracking effect. Lu et al. developed a Cu^{2+}-detecting proportional micelle based on hydrophobic carbon dots for biosensing and imaging of Cu^{2+} in hepatocytes. The probe has the characteristics of single wavelength excitation and dual emission, excellent selectivity, low detection limit and high sensitivity. In addition, the probe offers a way to track variations in Cu^{2+} levels in hepatocytes due to its small size, minimal cytotoxicity, outstanding biocompatibility, and strong cell permeability [5].

Photoacoustic imaging (PA imaging) is an advanced non-invasive multi-modal imaging technology. As a hybrid imaging technology, PA imaging has good spatial resolution and deep tissue penetration in optical and ultrasound imaging. Bao et al. used EDA and CA as raw materials and DMSO as the solvent to synthesize CDs by near-infrared radiation [6]. With a high photothermal conversion efficiency, the greatest absorption is measured at 600 nm, while the near-infrared high emission peak is measured at 720 nm. As biomedical reagents for tumor FL imaging, tumor PA imaging, and PTT, the CDs are safe to use.

3.2. CDs for Biosensing

Biosensors have received widespread attention in recent years. Specifically, nanostructure-based biosensors provide more sensitive detection due to their outstanding properties. Carbon dots are widely used in biosensing, and researchers have successfully designed and prepared various types of sensors for detecting and analyzing target substances.

Owing to its modest photodamage of biological tissues and high organ penetration depth, red emissive CDs have garnered increased attention. In order to create red emissive fluorescent CDs, Gao et al. described a straightforward one-pot hydrothermal process that uses ultrapure water as the solvent and citric acid (CA) and neutral red (NR) as the raw ingredients. In both the solid and aqueous states, the produced CDs exhibited red fluorescence, respectively. Under UV light, the CDs also show outstanding photostability, little toxicity, and biocompatibility. These CDs can be successfully applied in biosensing, as they have been shown to be effective in detecting Pt^{2+}, Au^{3+}, and Pd^{2+} noble metal ions [7]. Biosensing is a technique frequently employed in the diagnosis of cancer. Su et al. successfully prepared a novel hafnium-doped compact disc (HfCD). It is anticipated that HfCDs will be used for in situ CT/fluorescence imaging of hepatocellular carcinoma because of their many advantages, which include stability, biocompatibility, good solubility in water, outstanding performance as a CT contrast agent, and the potential to accumulate tumors favorably.

Moreover, combining nanomaterials with CQDs can enhance their functionality. Mehdipour et al. developed a sensitive and selective electrochemical detection sensor based on RGO/aunps modified electrodes for the detection of human prostate-specific antigen (PSA). This method can also be used for the detection of other related cancer biomarkers [9].

3.3. CDs for Disease Treatment

Numerous investigations have looked into the potential therapeutic uses of CDs for particular illnesses. Xia et al. synthesized red bean-based CDs and examined the anti-proliferative properties of these discs on several cancer cell lines, including colorectal, pancreatic, and liver cancer [10]. Their findings showed that CDs impeded cell migration in every cancer cell line tested. Moreover, doxorubicin and carbon dots together showed a stronger inhibitory effect on all cancer cell lines. Therefore, CDs may be alternative and complementary medicines for cancer treatment.

One non-invasive therapeutic approach is photodynamic therapy (PDT), which has gradually become the fourth line of choice in the field of cancer treatment after surgery, chemotherapy, and radiotherapy. PDT utilizes photosensitizers (PSs) that are activated under specific wavelengths of light. Reactive oxygen species (ROS) of many kinds can be produced as a result of these PSs' ability to transmit energy to the oxygen around them.These ROS can powerfully kill or induce apoptosis of tumor cells, thereby achieving the effect of treating tumors. Through reversible cross-linking processes, Chen et al. employed CDs to create new nanoscale covalent organic frameworks with significant reactive oxygen production capabilities, high water dispersibility, outstanding physiological stability, and strong biocompatibility. When taken up by cancer cells, it works wonders as a PDT medication for treating tumors. Experiments conducted in vitro and in vivo have conclusively shown how very effective PDT is at halting the formation of tumors and cell proliferation [11].

The quality of life of people with inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease (CD), is significantly impacted by this chronic, recurrent illness. IBD is characterized by and partially causes oxidative stress, which is brought on by unusually high amounts of ROS. Ma et al. used a solvothermal method to extract CDs nanozymes from glutathione and biotin to alleviate IBD [12]. We found that the obtained CDs nanozyme has good SOD enzyme activity and fluorescence ability. Research shows that CDs can eliminate excess ROS and effectively relieve colon inflammation. This CDs nanozyme has no obvious systemic toxicity and is expected to achieve long-term inflammation relief.

4. Challenges and Future Outlook

Due to their distinctive physical and chemical characteristics, excellent biocompatibility, and straightforward surface functionalization and doping, CDs have garnered considerable interest in the fields of bioimaging, biosensing, and tumor treatment. A number of significant advances have been made, ranging from the fundamental optical and physical properties of CDs to their potential applications. It is anticipated that carbon points will assume a more pivotal role in a multitude of fields in the future. Given the excellent biocompatibility of carbon dots, it is reasonable to posit that they will become an important biological application platform. Although significant advancements have been made, the practical application of carbon dots remains challenging and complex. One of the primary and critical safety issues associated with carbon dots is their biotoxicity. A number of studies have demonstrated that CDs have low cytotoxicity. However, significant safety concerns, toxicity, and pharmacological issues with colloids and metals have impeded advancements in cancer treatment and detection [13]. Although these problems won't stop CDs from being developed for in vitro applications, future in vivo uses will depend on the creation of more robust and non-toxic nanomaterials for the treatment of diseases in vivo.

5. Conclusion

The biomedical profession is reaching new heights thanks to the advancements and potential of carbon dots technology. With nanotechnology developing at a quick pace these days, carbon dots, a new nanomaterial, have shown a great deal of promise for use in the biomedical industry. The unique optical properties, good biocompatibility, and facile functionalization of carbon dots have enabled significant progress in the fields of bioimaging, sensing, and disease treatment. Nevertheless, the advancement of carbon dots technology is not without its challenges. One such challenge is the safety of carbon dots in biomedical applications, which requires further investigation. In addition, the advancement and future evolution of carbon dot technology necessitate interdisciplinary collaboration and communication to collectively advance the biomedical field. In conclusion, the innovation and future development trend of carbon dot technology will bring about revolutionary changes to the biomedical field. It is reasonable to posit that, through continued research and investigation, carbon dot technology will make a significant impact on human health in the future.

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