A Comprehensive Review of the Application of Carbon Dots as Vaccine Carriers and Adjuvants

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ABSTRACT

Developing next-generation vaccines (such as peptide, DNA, and mRNA vaccines) requires efficient delivery systems and potent immunogenic adjuvants to overcome the inherent limitations of these antigens' immunogenicity. Carbon dots (CDs) have attracted considerable attention in nanomedicine for their unique chemical structure, multiple functional groups, high stability, low toxicity, high biocompatibility and bioavailability, and cost-effective synthesis. This review article explores the dual role of carbon dots as "smart antigen carriers" and "immunogenic adjuvants." The mechanisms of antigen loading, protection from enzymatic degradation, facilitation of uptake by antigen-presenting cells (APCs), and activation of innate immune pathways (such as inflammasome activation and cytokine secretion) are discussed.

Keywords: *Carbon dot, Vaccine carrier, Adjuvant, system, Antigen delivery*

1. *INTRODUCTION*

Vaccination, one of the most promising preventive and therapeutic interventions for public health, plays a vital role in controlling and eradicating cancer and infectious diseases. However, the transition from traditional vaccines (live attenuated or killed vaccines) to next-generation vaccines (subunit, peptide, DNA, and mRNA) has created new challenges. Although these vaccines are safer, they often elicit weak innate immune responses and are rapidly degraded by enzymes in the body. Therefore, nanoscale delivery systems and adjuvants are needed to enhance humoral and cellular immune responses [1, 2]. Traditional adjuvants such as aluminum salts (Alum) primarily stimulate the humoral immune response (Th2) and fail to induce robust cellular immunity (Th1 or CTL), which is necessary to combat viruses and cancers. Meanwhile, carbon nanomaterials, especially carbon dots, have emerged as ideal candidates to overcome these limitations due to their unique physicochemical and biological properties.

Carbon dots are spherical nanoparticles less than 10 nm in size, first identified in 2004. Their key features making them suitable for vaccine use include: 1) They mainly consist of carbon, oxygen, nitrogen, and hydrogen, which are highly biocompatible with biological systems. 2) Their surface is rich in functional groups such as hydroxyl, carboxyl, and amine, allowing covalent or noncovalent attachment of antigens, targeting ligands, and adjuvants. 3) They are resistant to photobleaching and enzymatic breakdown, helping protect the antigen until it reaches the target cells. 4) Their fluorescent qualities allow for in vivo imaging and tracking of vaccine distribution within lymphoid tissues without the need for additional dyes.

In this article, we provide a comprehensive and up-to-date overview of the biomedical applications of carbon dots, particularly their use as vaccine adjuvants and carriers.

2. *Classification of carbon dots and synthesis methods*

The term "carbon dots" refers to a wide variety of fluorescent, nearly spherical nanoparticles, typically less than 10 nm in size. They differ markedly in crystallinity, composition, and synthesis methods [3]. CDs are typically divided into four main subtypes based on their structural composition and synthesis method: Carbon Polymer Dots (CPDs), Graphene Quantum Dots (GQDs), Carbon Nanodots (CNDs), and Quantum Dots (CQDs) (Fig. 1). GQDs are anisotropic particles made up of one or more graphene layers with distinct edge functionalities. CNDs are amorphous and usually show fluorescence from surface or molecular fluorophores. CQDs are roughly spherical with a graphitic or semi-crystalline core, while CNPs are spherical, amorphous particles. All types of CDs share surface functionalities, such as oxygen- and nitrogen-containing groups. There are two methods for synthesizing carbon dots: top-down and bottom-up. Top-down approaches primarily involve aggressive chemical or physical processes, such as laser ablation, to break down and destroy bulk carbon sources like graphite and carbon nanotubes, producing small carbon dots. In contrast, the bottom-up approach, including hydrothermal carbonization, pyrolysis, and microwave-assisted synthesis, primarily involves the polymerization and carbonization of small molecules into CQDs via chemical reactions [4-6].

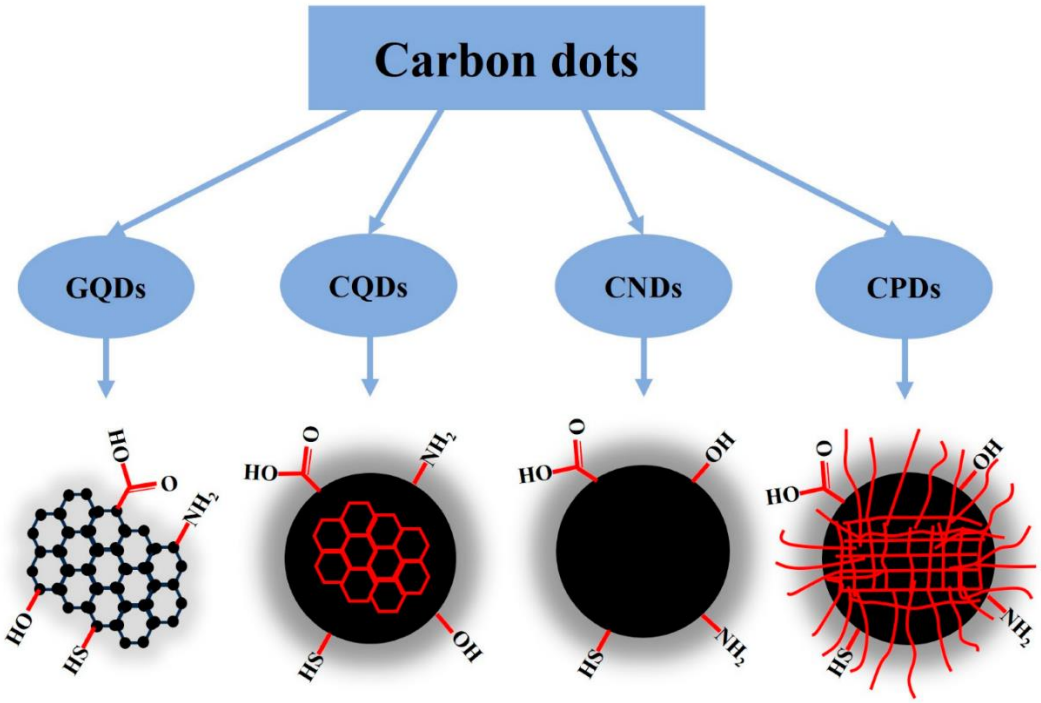


Figure 1: *Representation of the four main types of carbon dots. Graphene Quantum Dots (GQDs), Carbon Quantum Dots (CQDs), Carbon Nanodots (CNDs), and Carbon Polymer Dots (CPDs).*

3. Carbon dots as antigen carriers

The primary role of a carrier is to protect the antigen and to facilitate its entry into antigen-presenting cells (APCs), such as macrophages and dendritic cells (DCs) [7]. Antigens (proteins or nucleic acids) can be loaded onto the surface of CDs via electrostatic interactions, hydrogen bonding, or covalent binding. For example, positively charged CDs readily form complexes with negatively charged DNA or mRNA [8]. The small size of CDs (typically 2–10 nm) allows them to drain through lymphatic vessels and reach lymph nodes, the main sites of T- and B-cell activation. Due to their surface properties, CDs are readily engulfed by dendritic cells via endocytic pathways (such as micropinocytosis or clathrin-dependent endocytosis), which can prevent antigen escape into the endosome and, in some modified CDs, facilitate their release into the cytosol (the proton-sponge effect) [9].

4. Carbon Dots as Immunogenic Adjuvants

In addition to serving as antigen carriers, carbon dots can function as intrinsic adjuvants that stimulate the immune system. Studies have shown that GQDs can activate the NLRP3 inflammasome in macrophages by inducing mild oxidative stress. This triggers the processing and secretion of the interleukin-1 beta precursor (pro-IL-1β) into its active form (IL-1β), a key proinflammatory cytokine that initiates vaccine responses [10].

5. Applications of Carbon Dots in Vaccination

The potential of CDs for vaccination extends beyond theory and has been evaluated in numerous in vitro and in vivo studies. These studies are classified into four primary categories according to pathogen type or vaccine approach.

5-1. Vaccines against infectious diseases

The gp85 protein has been identified as a key immunogenic component of the ALV-J virion. Cheng Jia and colleagues synthesized carbon dots from humic acid, loaded them with gp85, and immunized chickens. Blood samples were collected weekly to measure serum antibody levels and viremia. The results showed higher antibody levels in the gp85-CQDs group than in the gp85–Freund's adjuvant group, with the difference statistically significant ($P < 0.05$). Viremia assessments indicated that the gp85-CQDs group experienced greater protection than the Freund's adjuvant group [12]. Chlamydia trachomatis causes ocular and genital infections, including blindness, urethritis, and cervicitis. Pgp3 is an important virulence protein encoded by the cryptic plasmid. Zhao et al. synthesized graphene oxide quantum dots to stabilize a Pickering emulsion (GQPE) and used this formulation as an adjuvant to enhance immune responses to the Chlamydia trachomatis Pgp3 recombinant vaccine. They immunized mice and investigated their immune responses and safety. Their research showed that GQPE effectively activates adaptive immunity by stimulating IgG, sIgA, IFN-γ, IL-4, and TNF-α, without causing toxicity to key organs such as the liver, kidney, and heart in mice [13].

5-2. Cancer Vaccines

The goal of cancer vaccines is to break immune tolerance and activate cytotoxic T cells (CD8+) against the tumor. Xu et al. developed a pH-responsive carbon dot from Lycium barbarum (Lyc-CDs) and loaded it with OVA to form Lyc-OVA nano-vaccines. They investigated the immuno-oncological effects of Lyc-CDs in a bilateral B16-OVA melanoma model and found that Lyc-OVA suppressed tumor growth by activating CD4+CD8+ T cells, reducing immunosuppressive Treg/MDSC populations, and modifying the tumor immune microenvironment [14]. Zhao engineered an aluminum-hybridized carbon dot (AH-CD) platform and designed a nano-vaccine, AH-TCL. This vaccine notably enhances lymph node (LN) targeting and stimulates dendritic cell (DC) maturation in draining LNs, achieving twice the effect compared to control vaccines. This robust immunostimulatory effect leads to the simultaneous suppression of primary and distant tumors through several mechanisms: (1) promoting DC maturation and infiltration into tumors; (2) boosting tumor-infiltrating CD8+ T cells by 1.9 times while decreasing immunosuppressive Tregs and MDSCs; and (3) increasing important proinflammatory cytokines such as TNF-α, IFN-γ, and IL-6. The high effectiveness of AH-TCL arises from aluminum doping, which enhances antigen presentation and lymphatic targeting, thereby activating both Th1 and Th2 immune responses to prevent metastatic spread [15]. Liu et al. found that antigen-loaded ZnMA offers multiple immunotherapeutic benefits by encouraging dendritic cell maturation and stimulating Th1-type cytokine production. Additionally, ZnMA promotes the synergistic expansion of CD8+ T cells and reduces Treg/MDSC cells, leading to near-complete tumor regression and restoring the tumor microenvironment's balance toward anti-tumor immunity [16]. Kim et al. developed an ionizable lipidoid called Cdoids, made by conjugating carbon dots (Cdots) with oleic acid, for nasal delivery of mRNA cancer vaccines. Using Cdoids in lipid-based nanoparticles improves mRNA vaccine stability and delivery efficiency for intranasal (IN)

administration, harnessing the nasal mucosal immune system to elicit robust systemic and local immune responses [17].

5-3. Mucosal Vaccines

Mucosal immunization (intranasal or oral) activates the first line of defense. Due to their extremely small size and the ability to be surface-modified with mucus-penetrating materials (such as chitosan or PEG coating), CDs can cross the mucus layer and reach M cells in Peyer's patches or nasal-associated lymphoid tissue (NALT). Hong Lei reported that in intranasal vaccine models, CCD/RBD-HR formulations elicit strong mucosal immunity, demonstrated by tissue-resident T cells in the lungs and airways. Furthermore, CCD/RBD-HR activates antigen-presenting cells, such as dendritic cells, effectively targets nasal epithelial cells, improves antigen binding via sialic acids, and, surprisingly, stimulates nasal epithelial cells to present antigens [18].

6. Conclusions

Carbon dots are a promising platform for next-generation vaccination systems because of their unique combination of biocompatibility, ease of surface engineering, and intrinsic immunostimulatory properties. They not only serve as efficient carriers for antigen protection and delivery but also act as potent adjuvants by activating innate immune pathways. Overcoming manufacturing standardization challenges and deepening understanding of their pharmacokinetics will pave the way for these nanomaterials to enter clinical trials and revolutionize the prevention of infectious diseases and cancer.

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