

Review

Not All Heroes Wear Capes—Some Just Glow: Exploring Carbon Dots as Nanoagents Against COVID-19

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Abstract

The coronavirus disease (COVID-19) pandemic highlighted critical limitations in ongoing diagnostic and therapeutic strategies, underscoring the urgent need for alternative and complementary approaches. Carbon dots (CDs) represent a novel class of fluorescent carbon-based nanomaterials with increased research interest owing to their enhanced biocompatibility, tunable photoluminescence, aqueous solubility, and rich surface chemistry. This review outlines current approaches in the diagnosis and treatment of COVID-19, along with the main structural, physicochemical, and biological features of CDs. Recent advances in the application of CDs as biosensing and antiviral nanomaterials against SARS-CoV-2 are critically discussed with an emphasis on the influence of synthetic routes, precursor compounds, heteroatom doping, surface functionalization, and purification strategies on their optical behavior and biological performance. CD-based biosensors mainly rely on fluorescence-sensing strategies, exploiting changes in photoluminescence intensity or energy-transfer processes upon recognition of viral targets. Electrochemical approaches are also investigated, highlighting the role of CDs as electrode modifiers to enable sensitive immunodetection in complex biological samples. Apart from diagnosis, the therapeutic potential of CDs is underscored by inhibiting viral entry, replication, disrupting cell membranes, or modulating oxidative stress. Finally, current limitations, challenges, and future perspectives regarding mechanistic understanding, synthesis parameters, biodistribution, and clinical translation are discussed, demonstrating the potential of CDs as novel “shiny weapons” against COVID-19, combining therapeutic and diagnostic functions.

Keywords: COVID-19; carbon dots; nanomaterials; nanomedicine; theranostics; detection; treatment



Academic Editor: Isabel Cansado

Received: 8 June 2026

Revised: 15 July 2026

Accepted: 21 July 2026

Published: 22 July 2026

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1. Introduction

Coronavirus disease 2019, commonly known as COVID-19, represents a highly infectious and potentially life-threatening disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus was initially identified in Wuhan, China in 2019 and subsequently disseminated globally, resulting in a pandemic. SARS-CoV-2 is

primarily transmitted through respiratory droplets, and typical symptoms include fever, cough, and breathing difficulties. The outbreak of COVID-19 has posed unprecedented challenges to every aspect of society, with public health being the most severely affected sector [1]. SARS-CoV-2 has infected millions of individuals worldwide, gravely affected healthcare systems, and resulted in thousands of lives globally. Apart from its direct health impact, the pandemic also caused a disruption in economic activity and daily life, creating an urgent need for effective diagnostic and therapeutic strategies [1,2].

From this perspective, nanotechnology has emerged as a promising approach in the management of COVID-19 during the pandemic and the post-pandemic era. Nanomaterials with a size limit of 1–100 nm and superior magnetic, chemical, optical, thermal, and mechanical properties have emerged as a revolutionary force in the field of biomedical science [3]. As shown in Figure 1, various categories of nanomaterials have been studied for sensitive and selective detection of viruses, as well as antiviral treatment [4]. Among them, carbon-based nanomaterials played a contributory role in combating COVID-19, owing to their non-toxicity, structural versatility, and chemical stability, supporting their application in sensors, personal protective equipment, vaccines, and antiviral drugs [1].

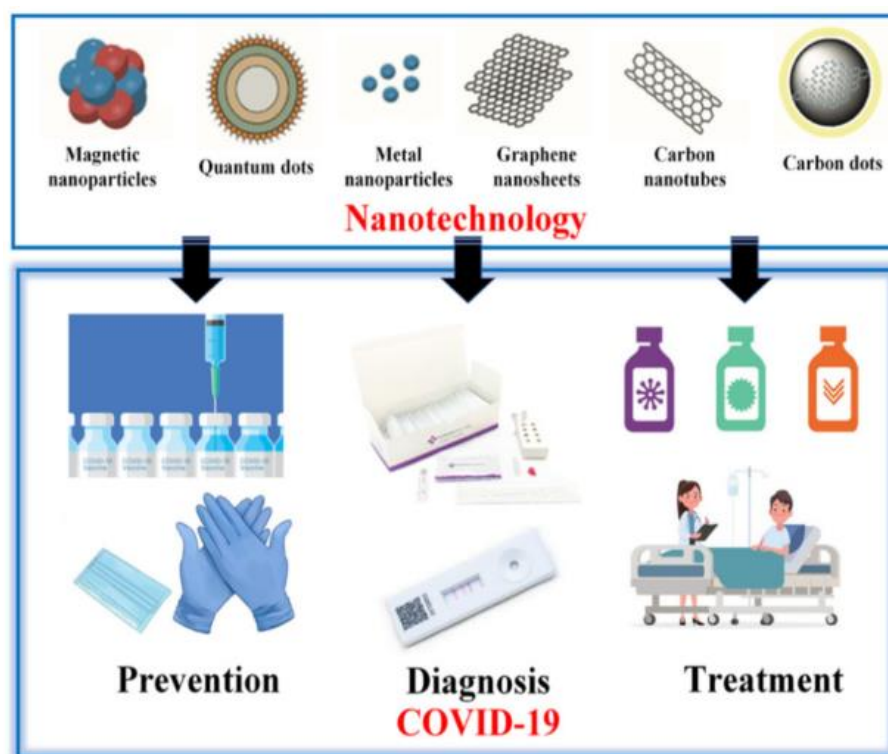


Figure 1. The role of nanotechnology in the management of COVID-19 (adopted with permission by [5]).

Carbon dots (CDs) are a new addition in the family of carbon-based nanomaterials that have attracted increasing research interest owing to their ultrasmall size, rich surface chemistry, and unique optical and electronic properties. CDs exhibit increased aqueous solubility, chemical stability, biocompatibility, and tunable photoluminescence (PL) [6,7]. These attributes, combined with low-cost and scalable synthesis routes, make CDs ideal candidates for biomedical applications, including bioimaging, drug delivery, biosensing, antimicrobial therapy, etc. (Figure 2). Additionally, the surface of CDs is abundant in functional groups, which render CDs water soluble and facilitate their interactions with other biomolecules, such as proteins, nucleic acids, etc. [8].

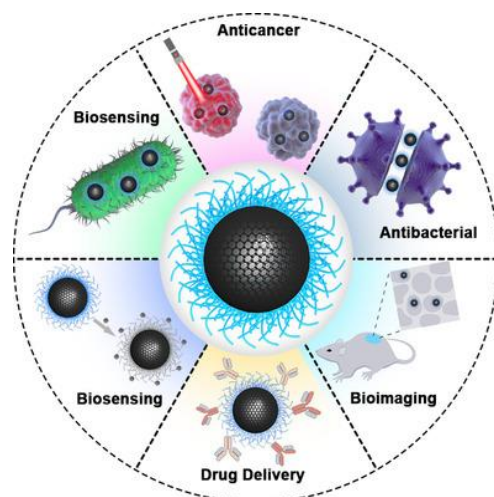


Figure 2. Overview of the main biomedical applications of CDs (adopted with permission by [9]).

Recently, CDs have shown remarkable potential in the detection of SARS-CoV-2, providing rapid, selective, and affordable alternatives to conventional diagnostic methods, such as Reverse Transcription Polymerase Chain Reaction (RT-PCR). Several CD-based biosensors employing fluorescence or electrochemical techniques have been developed for the detection of viral RNA, proteins, and other SARS-CoV-2-related biomarkers, enabling point-of-care testing (POCT) with high sensitivity and specificity. Beyond diagnostics, CDs have also emerged as potential antiviral agents towards COVID-19. Several studies have reported the intrinsic antiviral activity of CDs, which has been mainly attributed to their nanoscale dimensions, surface chemistry, and plentiful functional groups enabling interactions with key viral components. These interactions affect critical stages of the viral life cycle, such as viral attachment, entry, and replication processes, highlighting CDs as a novel weapon in the therapeutic armamentarium of COVID-19 [10–12].

This review summarizes recent advances in the utilization of CDs for the detection and treatment of COVID-19, with an emphasis on their synthesis strategies, functionalization approaches, sensing mechanisms, and antiviral action pathways. In particular, the fundamental characteristics of COVID-19 are initially discussed, as well as the current diagnostic and therapeutic strategies. Furthermore, the synthetic, structural, physicochemical, and biocompatibility aspects of CDs are presented, along with the recent insights into their application in COVID-19 detection and treatment. Finally, current challenges and future perspectives, including scaling-up, mechanistic understanding of CD–virus interactions, clinical translation, and the contribution of machine learning, are also discussed to highlight future perspectives for the development of CD-based nanoagents in the ongoing fight against COVID-19 and potential future viral pandemics.

2. COVID-19 Pandemic: Has It Come to an End?

The emergence of COVID-19 diseases caused by SARS-CoV-2 virus has fundamentally reshaped our understanding of pandemics, demonstrating the profound and complex challenges such global health crises can pose. Beyond its immediate public health impact, the pandemic has exposed vulnerabilities across economic, social, and scientific infrastructures [13]. Although the world is adapting to coexist with SARS-CoV-2, future outbreaks by its variants and other respiratory viruses are inevitable [14] and predicted to be among the top list of the causes of future pandemics [15]. By the end of 2025, the accumulative of confirmed deaths is over 7.1 million worldwide, according to the World Health Organization (WHO) [16]. Their ease of transmission routes and high mutation rates contribute to the rapid evolution of the virus as has been shown by the continued emergence of new variants

and subvariants that sometimes partially evade immunity from previous vaccinations and infections [17,18]. Therefore, continuous development in rapid detection, surveillance, and the improvement of treatments and vaccines is essential for future preparedness.

SARS-CoV-2 is an enveloped, positive-sense single-stranded RNA virus [19] with the infectious virion ranging from 80–120 nm in diameter. The infectious SARS-CoV-2 virion contains 29,903 nucleotide RNA genomes, which can encode for 25 non-structural and 4 structural proteins [20,21], the latter of which are nucleocapsid (N), membrane (M), and envelope (E), and they are decorated with crown-like structure spike (S) proteins (Figure 3) [22]. The high abundance of the N protein and the surface exposure of the S protein render them primary targets for viral detection and development of treatment and vaccine. The infectious virion spreads and enters the body through several routes. However, the most common route remains the respiratory track, which occurs primarily through aerosols from body fluids. Beyond the respiratory tract infection, facilitated by the S protein, different variants possess the ability to infect tissues and organs differently. As has been reported, the span of the clinical presentations includes neurological [23,24], cardiovascular, gastrointestinal [25], musculoskeletal, and immunological domains beyond the classical presentations, such as fever, persistent cough, fatigue, headache, and sore throat. This was confirmed by the detection of the virus in various organs and tissues, such as the heart, kidney, intestine, and liver [26–28].

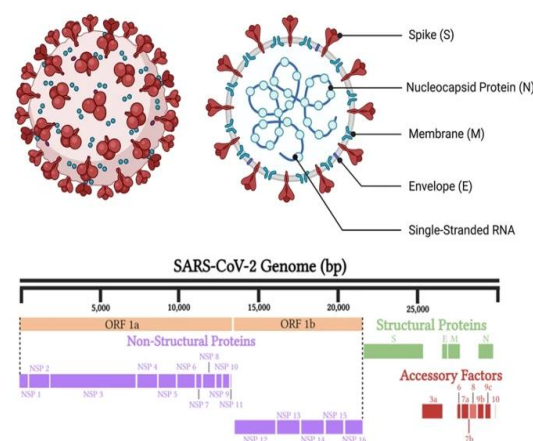


Figure 3. Structural elements of human SARS-CoV-2 (adopted with permission by [29]).

More recently, growing evidence (though still debated) indicates the persistence of SARS-CoV-2 infection within individuals, reflected by the so-called “long COVID”. Clinically termed Post-Acute COVID-19 Sequelae (PASC), this condition encompasses a heterogeneous constellation of chronic, often debilitating symptoms that persist well beyond the resolution of the acute infection [30–32]. Other plausible explanations include immune dysregulation, latent viral reactivation, or synergistic pathophysiology with other pathogens that introduce additional layers of clinical uncertainty and public health risk [33]. If the persistence of viral infection is the case of long COVID, the diagnostic or therapeutic strategy must consider both managing the newly emerged sub-variants and supplementing them with more sensitive and specific techniques to detect low levels of viral persistence, as well as using the more effective treatment against the persistent virus and their sequels to improve the patient outcomes.

3. COVID 19: An Overview in Current Diagnostic and Therapeutic Strategies

In response to the global threat, both molecular and immunological methods have been developed to screen the infected individuals. Molecular diagnosis primarily relies on the detection of the RNA genome of the virus. RT-PCR is a robust method with its

accurate ability in detecting RNA viruses in early infection (Figure 4). This technique allows for the detection of the specific sequence of both gene-encoding non-structural (RNA dependent RNA polymerase (RdRP)) and structural (N, E, and S) proteins of SARS-CoV-2 [34]. Although this molecular technique is considered as a high sensitivity and specificity technique, several challenges remain to be improved. RT-PCR can sometimes yield false-negative results, especially if the viral load is low or if the sample is not collected properly. PCR-based technique requires specialized laboratory equipment. This limits the accessibility for widespread use, including in remote or resource-limited settings. A simple and accessible rapid diagnostic test (RDT) has been developed (Figure 5). Unlike PCR-based techniques, this platform detects viral antigens, such as the N protein, which is present in a high quantity during active infection. This facilitates the rapid identification and isolation of the most contagious individuals. While RDTs offer significant advantages in terms of speed, ease of use, cost, and scalability, the issue on sensitivity is lower than PCR and often shows false negatives in the infected individual who carried low viral loads at the upper respiratory tract. Both molecular and immunological testing methods require further improvement, especially in light of the continual emergence of Omicron subvariants. Mutations in target regions can affect the binding efficiency of primers and probes, potentially leading to false negatives. This issue is particularly critical in cases of persistent infection or long COVID, where viral loads may remain low over extended periods.

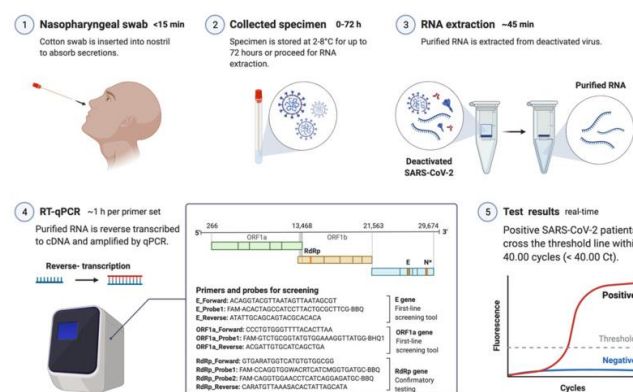


Figure 4. Schematic illustration of the RT-PCR diagnostic technique for COVID-19 (adopted with permission by [35]).

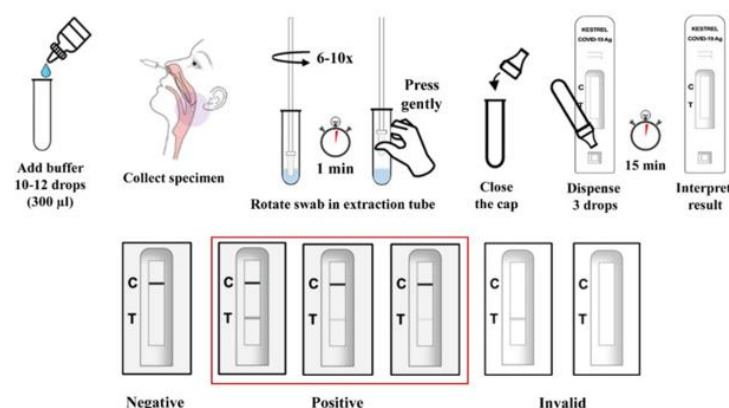


Figure 5. Schematic illustration of the rapid diagnostic test for COVID-19 (adopted with permission by [36]).

While societies may have psychologically moved on from the pandemic, SARS-CoV-2 remains biologically active, continuing to evolve and pose a threat. To date, no single antiviral therapy specifically targeting SARS-CoV-2 has been established. However, several

drugs have been approved or authorized by the Food and Drug Administration (FDA), WHO, and other health authorities, each targeting different stages of the viral life cycle. For instance, Remdesivir, a nucleotide analog, inhibits the viral RNA-dependent RNA polymerase (RdRp), thereby inhibiting viral RNA synthesis [34]. Molnupiravir (Lagevrio), a prodrug, introduces errors into the viral RNA genome during replication, leading to lethal mutagenesis of the virus [37]. Paxlovid is an oral antiviral that combines nirmatrelvir, an inhibitor of the main protease (Mpro, also known as 3CLpro or nsp5), which is essential for viral replication, with ritonavir, a pharmacokinetic enhancer that inhibits nirmatrelvir metabolism and thereby increases its systemic exposure [38]. In addition to these, several repurposed drugs have been explored for COVID-19 treatment, including Nitazoxanide [39], which was originally approved for protozoal infections, and Azithromycin [40], a broad-spectrum antibiotic. They have been investigated for their potential antiviral and immunomodulatory effects. Neutralizing antibodies have also been employed to prevent viral entry into host cells. These antibodies are essential as they directly block the interaction between the viral spike protein and the host angiotensin-converting enzyme 2 (ACE2) receptor; however, their effectiveness is increasingly challenged by the emergence of SARS-CoV-2 variants with spike protein mutations that reduce antibody-binding affinity [18,41]. Despite these therapeutic advances, challenges remain: the emergence of viral variants that can reduce drug efficacy [42], some antivirals require early administration within a narrow therapeutic window, and other antivirals pose risks of drug–drug interactions or limited bioavailability.

Taken together, the above limitations of current diagnostic and therapeutic strategies underscore the need for alternative approaches. Nanotechnology has emerged as a versatile platform capable of addressing multiple challenges. The unique physicochemical properties of carbon-based nanomaterials, such as carbon dots, render them promising tools in the management of COVID-19. Thus, the following sections explore the role of carbon dots in both diagnosis and treatment of COVID-19.

4. Introduction to Carbon Dots

4.1. Structure, Physicochemical Properties, and Synthesis Methods

In recent years, carbon-based nanomaterials have garnered the interest of the scientific community due to their unique properties, leading to their widespread application in diverse scientific and technological fields, such as biomedicine [8,43], energy storage and conversion [44], catalysis [45], electronics and optoelectronics [46], and environmental remediation [47]. CDs, the latest addition in the large family of carbon based-nanomaterials, are 0-D quasi-spherical nanoparticles with a size of <10 nm, consisting of a graphitic, amorphous, or semi-crystalline carbon core surrounded by various functional groups. As illustrated in Figure 6, various structures of CDs could be obtained, including: i. graphene quantum dots (GQDs), ii. carbon quantum dots (CQDs), iii. carbon nanodots (CNDs), and iv. carbon polymer dots (CPDs) [48]. Specifically, GQDs possess graphene layers with surface functionalities and edge defects with lateral dimensions significantly greater than their height. CQDs are quasi-spherical nanoparticles with a crystalline or amorphous carbon core, which is surrounded with plentiful surface functional groups. Both types of GQDs and CQDs exhibit the quantum confinement effect that defines their optical properties. On the other hand, CNDs are amorphous, quasi-spherical nanoparticles without exhibiting quantum confinement effect. CPDs are the most recently identified type of CDs, consisting of polymer and carbon structures with a carbonized core, along with polymer chains and surface moieties [7,49]. Their distinct structural differences arise from the broad range of available synthesis approaches.

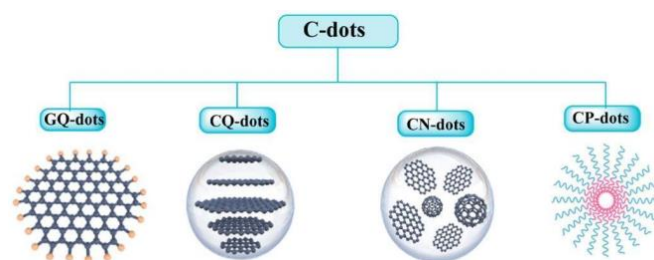


Figure 6. Classification of CD structure (adopted with permission by [48]).

CD synthesis can be classified into two principal approaches: top-down and bottom-up. Top-down methods involve the break-down of larger carbon-based structures (e.g., graphene, graphene oxide) into nanosized particles through physical or chemical processes. Among the most representative top-down methods are laser ablation [50], arc discharge [51], and chemical/electrochemical oxidation [52]. Although these methods can produce CDs with high crystallinity, they frequently require harsh reaction conditions, providing limited control over particle size and difficulties in heteroatom doping. On the other hand, bottom-up methods are based on the polymerization and carbonization of small organic molecules. Hydrothermal/solvothermal treatment [53], microwave-assisted synthesis [54], and pyrolysis [55] represent the most widely employed bottom-up techniques due to their simplicity, scalability, low cost, and ability to utilize environmentally friendly precursors. The selection of the synthesis method is crucial for the structural, physicochemical, and biological properties of CDs. Parameters, including precursor composition, reaction temperature, pressure, reaction time, and post-synthetic surface passivation influence particle size, crystallinity, surface chemistry, and PL of CDs [56].

Among other carbon-based nanomaterials, CDs have attracted considerable attention due to their small size, tunable surface chemistry, exceptional optoelectronic properties, and low or negligible toxicity, which make them potential candidates for sensing and antiviral applications. To begin with, the ultrasmall size of CDs (Figure 7a,b) represents a key factor governing their antiviral activity. At this scale, CDs possess a high surface-to-volume ratio, which improves their ability to interact with viral particles and biomolecules. Given that SARS-CoV-2 virion ranges from 80–120 nm, CDs can readily access and interact with its proteins or receptor binding domains (RBD) [1,57,58]. Size also represents an important parameter for the sensing abilities of GQDs, since PL of GQDs arises from quantum confinement effects, associated with their size [59]. In addition, CDs are well-known for their highly versatile surface chemistry; various functional groups (Figure 7c), including hydroxyl (–OH), carboxylic (–COOH), aldehyde (–CHO), amino (–NH₂), and thiol (–SH), can be incorporated onto their surface during synthesis process, depending on the choice of the precursors. In general, the nature and distribution of surface functional groups critically contribute to physicochemical properties, as well as antiviral performance of CDs [48]. As opposed to other carbon-based nanomaterials, CDs exhibit excellent aqueous solubility due to the presence of oxygen-containing functional groups in their structure. Moreover, the optical properties of carbon dots, including their exceptional PL and sensing capabilities, are strongly influenced by the fluorophores and surface functional groups generated during synthesis [59]. The surface chemistry of CDs plays a decisive role in mediating electrostatic interactions between them and the viral components. In particular, virus particles, including SARS-CoV-2, often display a negative charge as a result of phospholipids and amino acid residues [60]. Therefore, positively charged CDs (e.g., CDs that contain a large number of NH₂ functionalities) exhibit enhanced affinity toward viral particles via electrostatic interactions, inducing virus aggregation and inhibition of receptor-binding sites and thus leading to decreased pathogenicity [57,61]. Undoubtedly, the abundance of functional

groups enables the facile modification of CDs for the inhibition of several viruses [62]. Taking into account the role of triazole derivatives as effective inhibitors of SARS-CoV-2, triazole-modified CDs with increased antiviral activity and inhibition of the entrance of RNA viruses to cells have emerged as promising candidates. Similarly, CDs functionalized with phenylboronic acid have exhibited efficacy in inhibiting HIV infections. By binding to viral surfaces, benzoxazine-based CDs can impair virion functionality, thereby blocking the replication of adenovirus-associated viruses and parvoviruses during the initial phase of viral activity. Additionally, polyamine-functionalized CDs exhibit dose-dependent inhibition of White Spot Syndrome Virus infection by targeting and binding to the viral envelope [11,63].

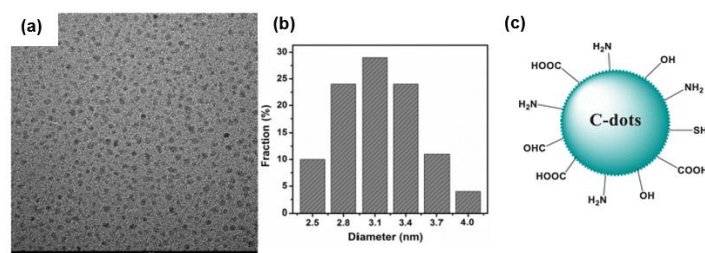


Figure 7. (a,b) TEM analysis and particle-size distribution of CDs (adopted with permission by [64]). (c) Abundance of functional groups onto the surface of CDs, depending on the precursor materials (adopted with permission by [48]).

As mentioned earlier, the strong and tunable PL of CDs is one of the most distinctive features of CDs, arising from a combination of surface states, quantum confinement effect, and molecular fluorescence. Among these factors, the surface state is considered the dominant mechanism, influenced by both the degree of surface oxidation and surface functional groups. Surface oxidation introduces defect sites that can trap excitons, often causing a red shift in emission (Figure 8a). In the case of similar oxygen content, the type and abundance of surface functional groups represent a crucial parameter in PL of CDs, further underscoring the key role of surface chemistry in tuning the optical properties of CDs. Another important parameter of PL is the quantum confinement effect of CDs, which is evident when the particle size approaches the Bohr radius. In this mechanism, the size of CDs, as well as the presence of aromatic domains, significantly influences their emission properties (Figure 8b). A further mechanism contributing to the PL of CDs is molecular fluorescence, which originates from fluorescent molecular species formed during bottom-up synthesis, such as imidazo[1,2-a]pyridine-7-carboxylic acid, 1,2,3,5-tetrahydro-5-oxo-(IPCA) [49]. The unique optical characteristics of CDs enable their application as fluorescent sensors for virus detection. In this aspect, CDs can bind with viral proteins or other biomolecules resulting in measurable changes in PL intensity or emission wavelength [65]. These factors also underpin the dual role of CDs as reactive oxygen species (ROS) generators and ROS scavengers, depending on synthesis parameters [66]. On the one hand, CDs can produce ROS upon light irradiation, enabling photodynamic inactivation (PDI) as an antiviral approach. In particular, CDs can produce ROS (e.g., $O_2^{\cdot-}$, $OH^{\cdot}$, 1O_2 , H_2O_2) with the aid of light and induce loss of virus infectivity by disrupting viral components [67]. On the other hand, CDs have been reported to act as ROS scavengers mitigating excessive oxidative stress and hindering inflammatory response [68]. Collectively, these physicochemical properties link the optical and chemical properties of CDs to both diagnostic and therapeutic applications against viruses.

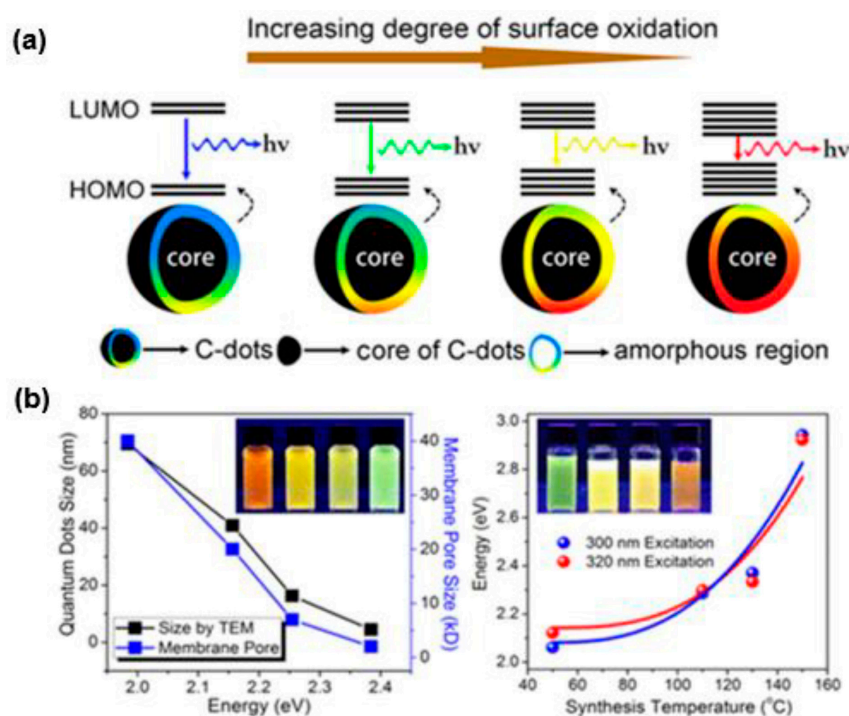


Figure 8. Tunable PL properties of CDs with varying (a) degree of surface oxidation (adopted with permission by [69]) and (b) size (adopted with permission by [70]).

4.2. Biological and Toxicological Aspects

Special optical features, surface functionality, and aqueous solubility promote the application of CDs as nanoagents against several viruses, including COVID-19. The investigation of potential industrial utilization of antiviral CDs raises many biosafety concerns, underlining the need for exploring their biocompatibility. Specifically, biocompatibility is an essential feature of any material involved in interactions with cells and living tissue. CDs are considered biocompatible materials compared to traditional semiconductor quantum dots (e.g., CdS, CdSe), taking into account the absence of toxic metal atoms and increased hydrophilicity that favors rapid clearance from the body [48,49,71]. Potential toxicity of CDs can be examined via a series of *in vitro* and *in vivo* techniques. For instance, *in vitro* assessment involves cells' incubation with several concentrations of CDs and assessment of cell survival compared to untreated cells with assays such as MTT, CCK-8, WST-1, etc. Another approach involves *in vivo* evaluation, in which CDs are administered directly into living organisms, such as mice or zebrafish, typically via intravenous or tail injection. The study of CD biodistribution to monitor their systemic circulation, organ accumulation, and potential toxicity is also an important aspect. Fluorescence imaging can reveal CD circulation in the bloodstream, their subsequent accumulation in the spleen and kidneys, and, finally, their clearance through renal filtration [48]. For instance, Chiu et al. [72] synthesized gadolinium–sulfur–nitrogen-co-doped CDs and studied their biocompatibility *in vivo* in zebrafish embryos. CDs did not affect the development of zebrafish embryos at any stage independent of the use of CDs in high concentrations or long incubation time with zebrafish embryos (Figure 9a). *In vivo* imaging revealed negligible autofluorescence in untreated zebrafish, whereas strong and homogeneous fluorescence was observed throughout the body after exposure to CDs, confirming efficient uptake and systemic distribution (Figure 9b). Biodistribution studies further showed pronounced fluorescence on day 1, followed by a gradual decrease over time, indicating progressive clearance of the CDs from the organism (Figure 9c).

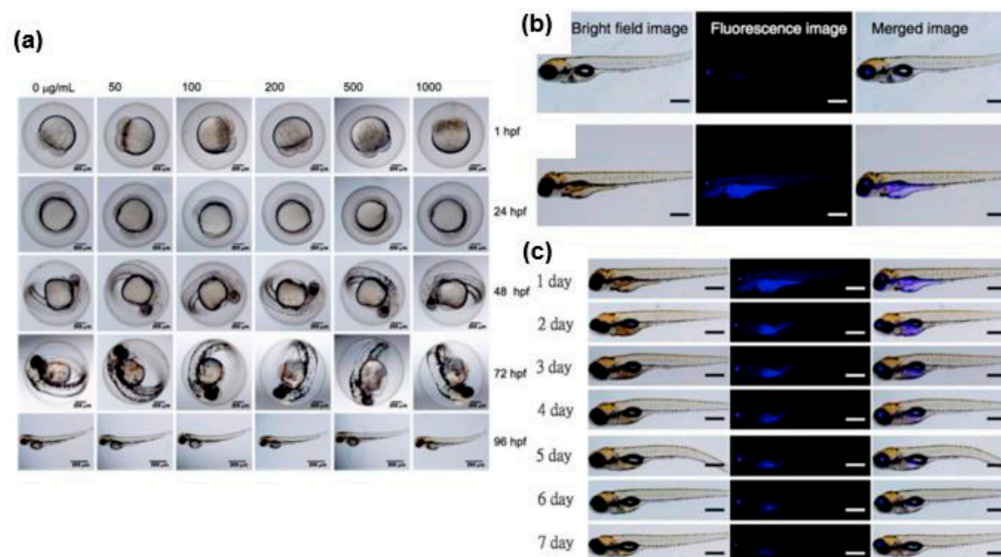


Figure 9. In vivo biocompatibility studies of CDs in zebrafish embryos. (a) Optical images of different developmental stages of zebrafish embryos upon exposure to varying concentrations of CDs. Bright-field fluorescence and merged images of zebrafish embryos (b) untreated (control) and CD-treated and (c) CDs-treated for various incubation periods of time. (Adopted with permission by [72]).

Blood compatibility is an additional parameter required for testing in materials employed for biomedical applications. The hemocompatibility of CDs can be assessed in vitro via several techniques, including hemagglutination, hemolysis, and coagulation behavior tests. A lot of parameters define the blood compatibility of CDs, such size, surface functionalities and structure [48]. The study of Li et al. [73] addressed the hemocompatibility of fluorescent CDs derived hydrothermally from α -cyclodextrin. As revealed in Figure 10, CDs did not affect morphology of red blood cells, and they did not cause lysis of red blood cells from CDs concentrations up to 0.1 mg/mL.

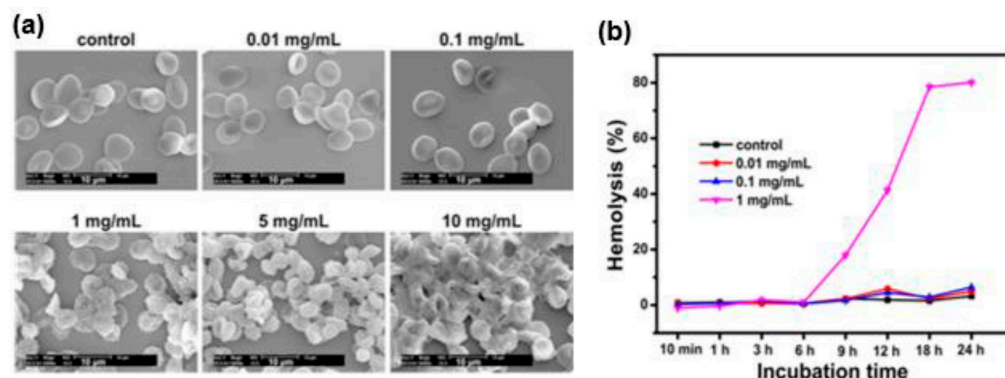


Figure 10. Blood compatibility investigation of CDs. (a) SEM images of red blood cells incubated with different concentrations of CDs. (b) Hemolysis of the red blood cells upon incubation with CDs in varying concentrations (adopted with permission by [73]).

5. Application of Carbon Dots in COVID-19 Detection

“Break the chain” became a global motto aimed to hinder the rapid transmission of COVID-19. Timely diagnosis of COVID-19 and subsequent isolation of infected individuals were the primary measures to eliminate the contagion of disease. The presence of viral particles at low concentrations in clinical samples, along with complex biological matrices, renders their detection challenging. These limitations underscore the need for the development of approaches and diagnostic tools capable of performing rapid, sensitive, and selective determination under laboratory and point-of-care (POC) conditions [1,57].

In this effort, biosensors have emerged as indispensable platforms to interrupt viral spread, providing accurate and reliable detection of virus biomarkers. By translating biological conditions into measurable optical or electrochemical signals, biosensors provide a promising alternative to conventional techniques such as PCR, which are often labor-intensive, time-consuming, and require sophisticated instrumentation [1,10,74]. The efficacy of biosensors relies on fluorescence changes via several mechanisms, including photoinduced charge transfer, energy transfer, electron transfer, etc. [57]. Based on this perspective, CDs are integrated into diverse sensing architectures, including fluorescent, lateral flow, and electrochemical platforms. The following section aims to present research studies, employing CDs.

5.1. Fluorescence-Based Detection of COVID-19 with CDs

Fluorescence-detection methods possess inherently high sensitivity, low detection limits, and rapid analytical response. Fluorescent CD-based biosensors have emerged as effective and versatile platforms in the detection of a wide array of COVID-19-related biomarkers and therapeutic agents. Unlike conventional fluorescent probes, the tunable PL and photostability of CDs allow for their use as fluorescent biosensors without the need for using complex immunorecognition elements [57,75,76]. These properties have been exploited in diverse sensing strategies, including metal-ion modulated fluorescence, aptamer-based recognition, nucleic acid hybridization, and drug-detection assays.

For instance, the study of Manadan-Anju et al. [75] underlines the significance of the development of a fluorescent probe capable of detecting neopterin (NPT). In particular, NPT is a metabolite generated by macrophages as a result of immunological response to COVID-19 infection. Since NPT content increases in COVID-19 patients, NPT can be considered as a valuable prognostic marker for the estimation of disease's severity. The development of an optical probe aims to overcome the issue of selectivity, which is evident in other NPT-detection approaches, including HPLC (High-Performance Liquid Chromatography) and electrochemistry. Sulphur, nitrogen-co-doped carbon dots (S,N-co doped CDs) were fabricated by a microwave technique, employing citric acid, urea, and thiourea as precursor molecules. S,N-co doped CDs were further doped *ex situ* with ferric ions (Fe-S,N-co doped CDs). This type of doping led to the quenching of S,N-co doped CDs, and their initial orange color turned into red with the addition of Fe^{3+} ions. Upon the presence of NPT, Fe^{3+} interacts with NPT, leading to its dissociation from the CD surface and subsequent fluorescence recovery. The proposed sensor provides linearity in the range of 0–10 nM and a LOD of 3.11 nM. The selectivity of the sensor towards NPT was conducted with solutions of potent co-existing molecules, such as creatinine, uric acid, hemoglobin, glucose, cholesterol, folic acid, vitamin B12, methionine, and ions in higher concentrations than those evident in biological samples. All of the above biomolecules quenched fluorescence, apart from a slight response recorded for creatinine, glucose, Zn^{2+} , and Na^{+} . Furthermore, interference studies were performed in solutions containing NPT, as well as the above molecules, resulting in the unobstructed detection of NPT. The potential use of probes in the real sample analysis was also investigated by spiking serum and urine samples with NPT. The probe exhibited sufficient recovery rates in the range of 96–104%. The development of a POC system based on the Fe-S,N-co doped CD probe could enhance its applicability. Under this point of view, filter paper was coated with the produced probe, and a turn-on response was evident in the presence of NPT, which further confirms qualitative NPT sensing.

Sheffield et al. [77] proposed a dual-color CD-based fluorometric sensor for the detection of SARS-CoV-2 RNA. In this study, yellow CDs (y-CDs) were produced based on the solvothermal reaction of citric acid and urea in DMF and blue CDs (b-CDs) were synthesized via the hydrothermal reaction of diammonium hydrogen citrate. Both CDs

were conjugated with antisense oligonucleotide (ASO) sequences via carbodiimide coupling. These sequences enable the simultaneous and specific detection of multiple regions within the SARS-CoV-2 nucleocapsid (N) gene. The detection mechanism relies on the aggregation-induced emission enhancement (AIEE) of ASO-conjugated CDs in the presence of SARS-CoV-2 RNA. In particular, the conjugation of ASO with the target sequence of SARS-CoV-2 induces CDs aggregation, which restricts the rotation of the surface functional groups of CDs. As a result, non-radiative energy dissipation is suppressed, leading to an enhanced fluorescence signal. Selectivity studies revealed that only the combination of CDs resulted in an enhanced fluorescence signal in the presence of SARS-CoV-2 RNA compared to MERS or control RNA, whereas individual use of ASO-conjugated b-CDs or ASO-conjugated γ -CDs showed non-specific responses. This behavior confirms that the dual-ASO strategy enables selective RNA-induced aggregation only when the target N-gene sequence is present. The proposed fluorometric sensor achieved a detection limit of 81 RNA copies per μL , highlighting its high sensitivity for amplification-free SARS-CoV-2 RNA detection. Furthermore, purification studies showed that specific species found in the two types of CDs account for the sensor's selectivity. While ASO-conjugated b-CDs retained at 10 kDa preserved selective aggregation toward SARS-CoV-2 RNA, removal of these components (20 kDa cut-off) led to loss of selectivity and increased cross-reactivity with MERS RNA.

The study of Kannouma et al. [78] presented the application of CDs as fluorescent probes for the detection of the COVID-19 antiviral drug molnupiravir. The development of a fluorescent probe for the determination of this drug is an alternative solution to the commonly used chromatographic techniques, which cause an environmental burden due to organic solvents waste. This study proposed an environmentally friendly approach for the synthesis of CDs by using eggshell waste as the precursor (Figure 11). In particular, they produced CDs through a facile microwave-assisted pyrolysis of EDTA and chicken eggshell, which is abundant in elements and thus can facilitate the doping of CDs. The determination of the molnupiravir with the CDs is based on the quenching of the blue fluorescence of CDs in the presence of the drug. It is important to note the fact that the changes in CDs' fluorescence were quantitatively related with the concentration of the molnupiravir. The underlying mechanism of the proposed sensor is the static quenching through the formation of a non-fluorescent complex among the drug and the plentiful surface functional groups of CDs via hydrogen bonding and electrostatic interaction. The formation of this complex reduces the available free CDs, and thus a decreased fluorescence intensity is recorded. The examined sensor exhibited increased selectivity towards molnupiravir since no fluorescence quenching was recorded in the presence of excipients, such as HPMC, CMC, sorbitol, mannitol, glucose, etc. Additionally, researchers studied the efficacy of the CDs in the quantification of molnupiravir in hard capsules compared to HPLC. Results indicated no statistically significant difference between the two methods. Moreover, the developed fluorescent sensor based on CDs exhibited comparable LOD ($0.8 \mu\text{g}/\text{mL}$) and linear range ($2.5\text{--}70 \mu\text{g}/\text{mL}$) with the corresponding chromatographic techniques.

Among the various fluorescence-based sensing strategies, Förster resonance energy transfer (FRET) has been widely adopted due to its ability to translate nanoscale molecular interactions into measurable optical signals with high sensitivity. FRET requires a spectral overlap between the donor's emission and the acceptor's absorption. The distance between the donor and acceptor is a critical parameter for FRET efficiency [79,80]. The study of Mahani et al. [79] addressed the FRET phenomenon among CDs and gold nanoparticles (Au NPs) for the determination of the cytokine interleukin-6 (IL-6). Specifically, increased levels of this cytokine are highly associated with various disorders, including COVID-19 disease. Sensing of IL-6 is a challenging process given the facts that cytokine levels in

bodily fluids are typically very low and interference molecules may also be evident. An approach to deal with these conditions includes the use of nucleic acid aptamers, which present increased affinity towards IL-6. In this case, AuNPs were modified with a DNA aptamer, and nitrogen-doped CDs (N-CDs) were produced hydrothermally, using citric acid and ethylenediamine (EDA) as precursor compounds. In the absence of IL-6, there is a close spatial relationship between N-CDs and AuNPs, and N-CDs act as a donor, providing their energy to AuNPs, and this leads to fluorescence quenching. However, the FRET phenomenon is obstructed in the presence of IL-6, since IL-6 is selectively linked to the aptamer, resulting in the release of N-CDs from AuNPs/aptamers and recovery of N-CDs' fluorescence. It is important to note that an increase in N-CDs' fluorescence is highly related to IL-6 concentration. The proposed aptasensor exhibited linearity over the concentration range from 1.5 to 5.9 pg/mL with a LOD of 0.82 pg/mL, and these results were considered comparable with other fluorescent IL-6 sensors. Furthermore, IL-6 could be detected efficiently without interfering with signals from other molecules, including bovine serum albumin (BSA) and TNF- α . Experiments with the examined aptasensor were also performed in human serums samples, showing IL-6 recoveries between 95.7 and 102.9%. These results underline the potential of this FRET-based aptasensor using N-CDs and AuNPs in COVID-19 diagnosis with low cost and increased selectivity and specificity.

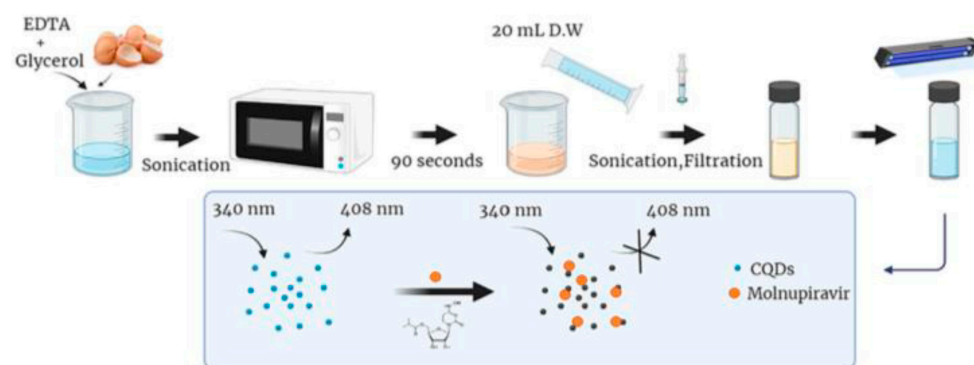


Figure 11. Schematic representation of the fabrication of CDs based on the microwave-assisted pyrolysis of eggshell waste and the development of a fluorescent probe for the quantification of the COVID-19 antiviral drug molnupiravir (adopted with permission by [78]).

When fluorescence-based sensing approaches are combined with immunorecognition elements, such as antibodies, highly selective fluorescent immunosensors can be obtained. In this context, FRET-based architectures are particularly advantageous, as antigen–antibody binding events directly modulate the donor–acceptor distance, leading to quantifiable fluorescence changes [81]. For instance, a biosensor was developed based on CDs and Au NPs pairs for the detection of S protein [82]. Au NPs are applied in SARS-CoV-2 detection due to their catalytic and electronic properties, as well as their biological inertness. In particular, AuNPs were positively ($^+$ AuNPs) or negatively ($^-$ AuNPs) charged, and anti-SARS-CoV-2 spike RBD-neutralizing antibodies (Ab) were conjugated with commercially obtained CDs, as well as with Au NPs. CDs/Abs act as energy donors, and Au NPs act as energy acceptors. In the presence of the S protein, immune reaction among antigens and antibodies takes place, resulting in a rise in the distance between donor and acceptor. Thus, the FRET phenomenon is hindered. Results showed the following fluorescence increase rate for the pairs $^-$ AuNPs/CDs–Ab, $^+$ AuNPs/CDs–Ab, $^-$ AuNPs–Ab/CDs–Ab, and $^+$ AuNPs–Ab/CDs–Ab: 6.8%, 18.6%, 9.5%, and 9.9%, respectively. Taking into account the negative charge of the CDs/Ab, FRET efficiency is enhanced for $^+$ AuNPs as a result of improved adsorption. Additionally, LOD values of 1.82 ng/mL, 0.05 ng/mL, 0.19 ng/mL, and 0.12 ng/mL were recorded for $^-$ AuNPs/CDs–Ab, $^+$ AuNPs/CDs–Ab,

$^{-}$ AuNPs-Ab/CDs-Ab, and $^{+}$ AuNPs-Ab/CDs-Ab, accordingly. When AuNPs were conjugated with antibodies, their surface charge became dominated by the negatively charged Ab, regardless of their initial charge. This reduced electrostatic attraction between AuNPs-Ab and QCDs-Ab increased donor-acceptor distance, resulting in decreased FRET. Therefore, $^{+}$ AuNPs/CDs-Ab exhibited the optimal performance in detection of the S protein with a linear range of 0.1–100 ng/mL and a total detection time of 12 min. These results are comparable with other methods detecting the S protein, and the proposed method has potential for large-scale applications. The detection of the S protein was tested in human serum samples with increased recovery rates regardless of the existence of other interfering compounds. Additionally, the biosensor was efficient in the detection of other COVID-19 variants, such as the Omicron variant (B.1.1.529) and the Delta variant (B.1.617.2).

The determination of serum-specific antibodies is an alternative option to diagnose COVID-19 infection, since it is a facile process and it mitigates the risk for “false negative” results. Under this point of view, fluorescence immunoassay has been proposed as an approach for the detection of COVID-19 antibodies (2019-nCoV mAb). The combination of several nanomaterials is encouraged in the development of fluorescent immunosensors in order to control the optical properties. Based on this perspective, Li et al. [81] fabricated a fluorescent probe based on QCDs and Ag@Au NPs. In this study, pyrolysis of citric acid was employed for QCD synthesis. The main role of Ag@Au NPs is to quench QCDs' fluorescence via the FRET mechanism. In particular, QCDs and Ag@Au NPs act as donors and acceptors, respectively, given their optical properties. Another important condition for FRET is the close distance between the donor and acceptor. When the 2019-nCoV antigen (NCP antigen) was combined with Ag@AuNPs, QCDs' fluorescence is still reduced despite the increase in distance. However, in the presence of 2019-nCoV mAb, the steric-hindrance effect takes place and distance among the nanosystem (antigen) and antibody increases, resulting in the increase of QCDs' fluorescence (Figure 12a). Ag@Au NPs also aim to reinforce the stability and the dispersion of the as-developed system. Some experimental conditions were optimized, including ratio of Ag and Au, NCP antigen concentration, and incubation time, as well as the pH and incubation time between antigen and antibody. Fluorescent detection of 2019-nCoV mAb was feasible since QCDs' fluorescence increased by increasing 2019-nCoV mAb concentration with a linear range of 0.1 pg/mL–10 ng/mL and a LOD of 50 fg/mL. As the 2019-nCoV mAb concentration rose, its combination with Ag@Au NPs was increased, so QCDs and Ag@Au NPs separated and QCDs' fluorescence was restored (Figure 12b). The examined nanosystem could efficiently detect 2019-nCoV mAb with increased selectivity and without interference from biomolecules, such as BSA, glutathione (GSH), L-cysteine, glucose, etc. (Figure 12c). The fluorescent probe exhibited sufficient stability even for 15 days upon storage in the refrigerator at 4 °C, resulting in the maintenance of fluorescence intensity up to 97%. Reproducible results were recorded upon the use of various batches of the probe for the sensing of 2019-nCoV mAb. Furthermore, experiments were conducted in artificial serum, further verifying its efficiency towards complex biological systems.

Wibrianto et al. [83] proposed the use of N, B co-doped CDs as markers for COVID-19 antibodies, such as immunoglobulin-G (IgG) and immunoglobulin-M (IgM). The levels of these antibodies highly rise upon infection with COVID-19 disease and are recognized as crucial indicators for the determination of the disease's state. Apart from this, determination of IgG and IgM is considered important for the assessment of vaccines against COVID-19. The enzyme-linked immunosorbent assay (ELISA) is a common technique implemented for the quantification of these antibodies, but it is labor-intensive, requires an extended duration to complete, and lacks sensitivity. Therefore, researchers investigated alternative detection methods, including fluorescence techniques [74]. CDs were produced by the pyrolysis of

citric acid and 2-aminophenyl boronic acid (APBA). The role of APBA was to introduce boronic acid groups capable of binding to cis-diol structures, which are present in IgG and IgM antibodies. Aim of this research was to investigate absorbance and fluorescence changes of CDs in the presence of these antibodies. The marker was tested in real human plasma samples, divided into the following categories with the use of a commercial rapid antibody test: K1 (IgG⁺/IgM⁻), K2 (IgG⁻/IgM⁺), K3 (IgG⁺/IgM⁺), K4 (IgG⁻/IgM⁻; pre-pandemic control), and K5 (IgG⁻/IgM⁻; pandemic-period samples). Results proved that CDs could bind to the antibodies in the blood plasma, since increased absorbance and fluorescence were recorded for samples in K1, K2, and K3 categories. Samples in the K4 category showed minimal absorbance and fluorescence, which is consistent with their status as negative controls. On the other side, samples of the K5 category showed moderately elevated fluorescence compared to the K4 category, suggesting the presence of low-level or previously undetected antibodies from rapid tests (Figure 13). Principal component analysis (PCA) further confirmed the results and highlighted the increased specificity of the proposed marker towards IgG antibodies, with a sensitivity of 74% and specificity of 53%. In the case of IgM detection, sensitivity of 63% and specificity of 27% were recorded based on PCA analysis.

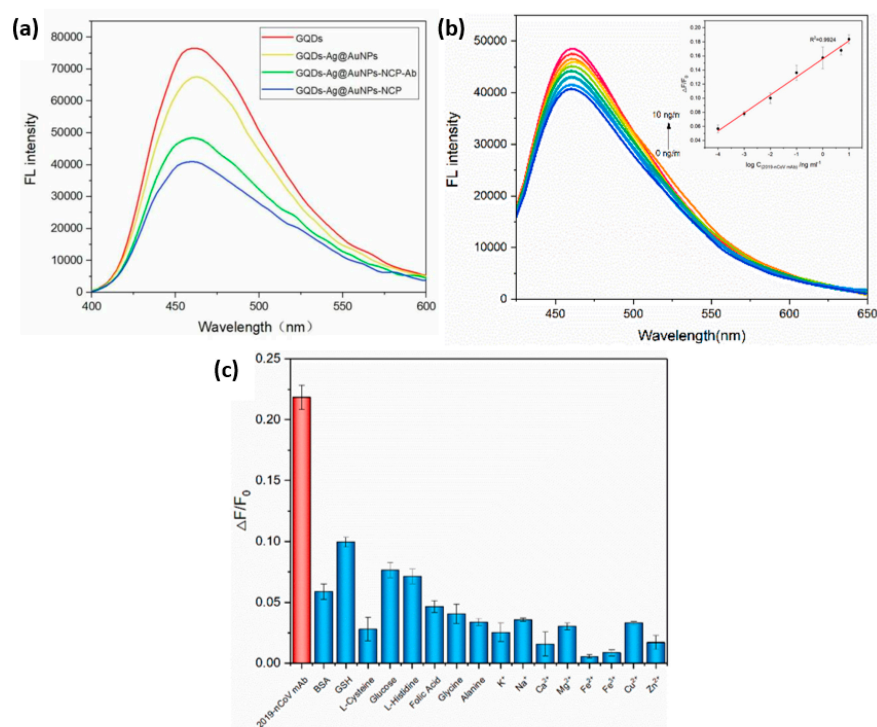


Figure 12. Fluorescence spectra of (a) GQDs, GQDs-Ag@Au NPs, GQDs-Ag@Au NPs-NCP, and GQDs-Ag@Au NPs-NCP- 2019-nCoV mAb, (b) GQDs and Ag@AuNPs-NCP mixture upon the addition of 2019-nCoV mAb in varying concentrations. Inset presents the correlation among the fluorescence increase efficiency and the logarithm of 2019-nCoV mAb concentration. (c) Selectivity of the proposed system towards 2019-nCoV mAb in serum (adopted with permission by [81]).

Apart from fluorescence intensity-based methods, ratiometric optical sensing is an alternative approach adopted to improve analytical reliability, selectivity, sensitivity to instrument fluctuations, and resistance to background light. This type of sensor is based on the detection of intensity changes of two or more fluorescence emission bands in response to specific analytes. Hamd-Ghadareh et al. [80] developed a radiometric fluorescence biosensor based on the FRET between CDs and AuNPs for the determination of N protein, which is considered an evolutionarily preserved biomarker of COVID-19 disease. In this case, dual-emissive CDs were produced based on the hydrothermal treatment of dill

leaves, proposing the use of alternative biomass-sourced precursors. The synthesized CDs exhibited dual emission at 475 and 675 nm upon excitation at 400 nm. Additionally, CDs were enclosed in SiO₂ nanocapsules (SNCs), aiming to improve sensitivity and the fluorescence signal by avoiding potential quenching from neighbor particles. The surface of the SNCs was functionalized with amine groups in order to immobilize anti-N protein antibodies. This type of antibodies was also immobilized into the surface of AuNPs. In both cases, the immobilization of antibodies secured the performance of the antibody–antigen reaction, when the N protein was present. CDs could perform their dual emission when they were released from the CDs@SNC core-shell structures with the aid of the reducing agent NaBH₄. In the presence of the N protein, an immunocomplex is formed between the N protein, CDs, and AuNPs, forming a sandwich structure. In this case, the N protein acts as a bridge among CDs and AuNPs since it interacts with the immobilized antibodies and FRET occurs. In particular, an increase in the N protein's concentration leads to an increased fluorescence ratio of I_{475}/I_{675} because emission intensity at 675 and 475 nm decreases and remains stable, accordingly. The developed probe could be used as a sensor with a LOD of 2 pg/mL in the range of 0.01–5 ng/mL, while sensitivity was recorded 54 times higher in comparison with other immunoassays, employing CDs. This probe exhibited enhanced selectivity towards the N protein's sensing even in the presence of CA125, CA15-3, CA19-9, bilirubin, and BSA, paving the way for further use in actual samples. The determination of N protein was examined in human serum samples, resulting in recovery rate up to 106%.

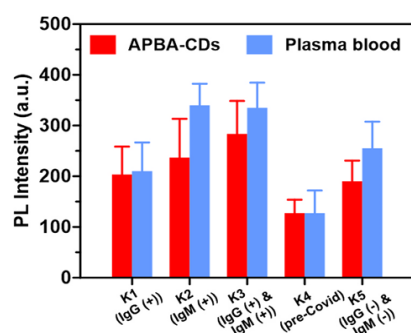


Figure 13. Fluorescence of various human serum samples with and without CDs, using excitation wavelength of 360 nm [83].

5.2. CDs-Based Lateral Flow Immunoassay for the Detection of COVID-19

Some other studies propose the use of CDs for SARS-CoV-2 detection via fluorescent lateral flow immunoassay (LFIs). This approach is considered as a POC diagnostic method and is characterized by convenience, efficiency, and low detection time. By integrating CDs into a strip-based format, these assays combine the high sensitivity of fluorescent detection with the simplicity and portability of lateral flow devices. Biocompatibility, aqueous solubility, and superb optical features of CDs render them particularly suitable as fluorescent labels in LFIs, enabling stable signal output and enhanced analytical performance. As a result, CD-based fluorescent LFIs provide rapid and user-friendly platforms for the detection of SARS-CoV-2 antigens or antibodies [10,74,84].

Xu et al. [84] were focused on this detection strategy and fabricated fluorescent nanobeads based on CDs that target nucleocapsid proteins. This type of protein is over-expressed during the infection and includes the genetic material. In nanobeads, a sphere is either surface coated with a fluorescent nanomaterial, or a fluorescent nanomaterial is encapsulated on the sphere. The aim of nanobeads is to generate fluorescence under the stimuli of external energy. In this case, red-emissive CDs were produced upon the solvothermal reaction of amidinothiourea and citric acid and the subsequent treatment with NaOH and HCl to reinforce the existence of carboxyl groups. Then, the carboxylic

groups of CDs were linked with the amino terminal of the solvent N- β -(aminoethyl)- γ -aminopropyltrimethoxy (AEAPTMS). This solvent can further boost its red emission, and its other side was linked with silica spheres. The product was finally coated with (3-aminopropyl) triethoxysilane (APTES), and CDs@SiO₂ spheres were formed. The proposed spheres were conjugated with the labeled antibody of SARS-CoV-2 nucleocapsid proteins (SARS-CoV-2 NP) for the development of immune spheres. LFIA strips were constructed for the evaluation of the detecting ability of the proposed system. In particular, the LFIA strip consisted of an absorbent pad, a sample pad, and the nanocapsule membrane, which included a control line coated with goat antimouse IgG and a test line with the coated antibody against SARS-CoV-2 NP. For the detection studies, immune spheres were mixed with a standard solution of SARS-CoV-2 NP based on an antigen–antibody reaction and then were dropped to the sample pad, followed by transfer to the absorbent pad. This led to the formation of a structure with the antibody of the test line and the surplus immune RCS spheres were bound with IgG of the control line. So, strong fluorescent signals were recorded, since both control and test lines possessed the spheres. In the absence of the analyte, only the control line had accumulated spheres, and therefore, only the control line showed a fluorescence signal. The fluorescence was examined by both techniques, including either a UV lamp or a fluorescent microscope. The microscope could achieve excitation by green light, which is preferred to achieve the red emission of the spheres. The application of UV lamp and fluorescent microscope especially resulted in sensitivities of the examined LFIA technology of 100 pg/mL and 10 pg/mL, respectively. Additionally, results were reproducible and conjugates maintained their stability at least 6 months of storage. It is important to note the fact that this structure between the test line and the conjugates was feasible only in the antigen of SARS-CoV-2 NP. No fluorescent detection was possible for other antigens, including AFP, ZiKa, SFTSV, and CEA, further underlining the strong selectivity of the proposed LFIA technology based on CDs. Human serum samples spiked with various concentrations of SARS-CoV-2 NP were employed to investigate the efficacy of the proposed technology with the use of fluorescent microscopy. In this case, high recovery rates of 96.97% to 102.80% were reported.

Several researchers focus on the detection of SARS-CoV-2 antibodies IgG and IgM with LFIAs. The study of Wang et al. [74] proposed the development of an accurate and low-cost LFIA strip based on SiO₂@Au@CDs to determine the antibody IgG (Figure 14). In particular, Au NPs were employed due to their increased localized surface plasmon resonance, biocompatibility, and facile functionalization, whereas SiO₂ NPs are known for their stability and their role as a carrier in various applications. The surface of SiO₂ was initially coated with Au NPs, and they were further linked with commercially obtained CDs, using PEI as a linker. Then the SiO₂@Au@CDs NPs were used to label the SARS-CoV-2 S1 protein, which is selective to antibodies against COVID-19. The resulting fluorescence was measured by a miniaturized fluorimeter, which was equipped in the strip. The proposed LFIA strip exhibited increased specificity towards serum samples positive in IgG antibody with improved detection characteristics in terms of linear range (7.4×10^{-7} to 7.4×10^{-4} mg/mL), LOD (1.2×10^{-7} mg/mL), and recovery rate compared to SiO₂@CDs strips. The addition of Au NPs facilitated fluorescence resonance energy transfer with the CDs and thus caused a rise in fluorescence. Additionally, Au NPs reinforced the specific surface area of SiO₂ NPs. The selectivity of the SiO₂@Au@CDs NPs-LFIA strip towards IgG was further investigated, using a mixture of numerous antibodies, including MERS-CoV IgG, RSV IgG, ASF1B IgG, and anti-SARS-CoV-2 IgG. Results showed increased fluorescence in the case of the anti-SARS-CoV-2 IgG antibody. It is important to note the fact that the maximum fluorescence signal was evident in 15 min, highlighting the speediness of this approach.

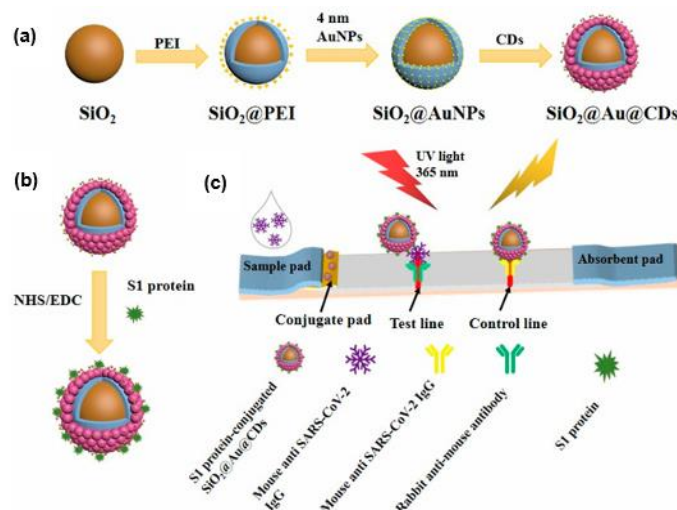


Figure 14. Schematic illustration of (a) the synthesis of SiO₂@Au@CDs NPs, (b) the as-prepared SiO₂@Au@CDs NPs labeled with S1 protein, (c) the development of LFIA strip (adopted with permission by [74]).

Ju et al. [10] proposed the use of aggregation-induced emission (AIE)-CDs for the detection of both IgG and IgM SARS-CoV-2 antibodies with an LFIA. AIE-CDs are promising candidates for LFIA applications because they exhibit enhanced fluorescence upon aggregation, in contrast to conventional carbon dots, which suffer from aggregation-caused quenching (ACQ). In this case, AIE-CDs were produced based on the solvothermal reaction of thiourea and citric acid, resulting in dual-fluorescence emission in blue and red. AIE-CDs were further conjugated with the labeled antibody of the SARS-CoV-2 spike protein (SSP) through a classical carbodiimide-coupling reaction. In the presence of IgM and IgG antibodies in the sample, a sandwich immune structure compound is formed with the AIE-CD-SSP located in the sample pad and with the mouse anti-human IgM/IgG antibody, immobilized on the test lines (T-lines) in the NC membrane. In parallel, during the migration of the specimen the AIE-CDs—chicken IgY located in the sample pad is caught by the goat antichick IgY, immobilized in the control line (C-line). This results in blue and red fluorescence in the T-line and C-line, respectively, upon blue and green irradiation. In the absence of IgM/IgG antibodies in the sample, only C-line fluoresces, indicating the validity of the strip. Figure 15a–c depicts the results of detecting IgM and IgG antibodies in various concentrations, ranging from 100 pg/mL to 100 µg/mL, employing the fluorescent marker AIE-CDs-SSP and fluorescence microscopy under blue and green light irradiation. It is important to note that fluorescence intensity was highly related to the concentration of SARS-CoV-2-antibodies. Additionally, the proposed sensor was reliable, since the ratio of dual emissions remained constant, serving as an internal standard to verify its performance. The selectivity of the proposed test towards SARS-CoV-2 S IgG was evaluated in the presence of Influenza A and Influenza B, resulting in fluorescent bands of T-line only for SARS-CoV2 due to the formation of immune structure (Figure 15d).

5.3. Electrochemical Detection of COVID-19 Based on CDs

Some other cases propose the use of electrochemical techniques for the detection of COVID-19 due to their simplicity, selectivity, portability, low cost, rapid response, and potential for POC diagnostics. In this approach, electrochemical immunosensors have gained research interest, as they combine the high specificity of antigen–antibody interactions with the sensitivity of electrochemical signal transduction. Compared to conventional detection techniques, electrochemical immunosensors provide simplified instrumentation, faster analysis, and the possibility of label-free detection, making them

suitable for facile and efficient detection. The performance of electrochemical sensors highly relies on the properties of electrode. Therefore, researchers are focused on modifying the surface of the electrode with nanomaterials so as to improve electron transfer, increase the active surface area, and enhance biomolecule immobilization. Among the various nanomaterials investigated, CDs have shown remarkable potential due to their excellent electrical conductivity, chemical stability, and ease of functionalization. Additionally, CDs can act as efficient signal amplifiers by facilitating charge transfer processes at the electrode–electrolyte interface, thereby significantly improving the sensitivity and reliability of electrochemical immunosensors for COVID-19 detection.

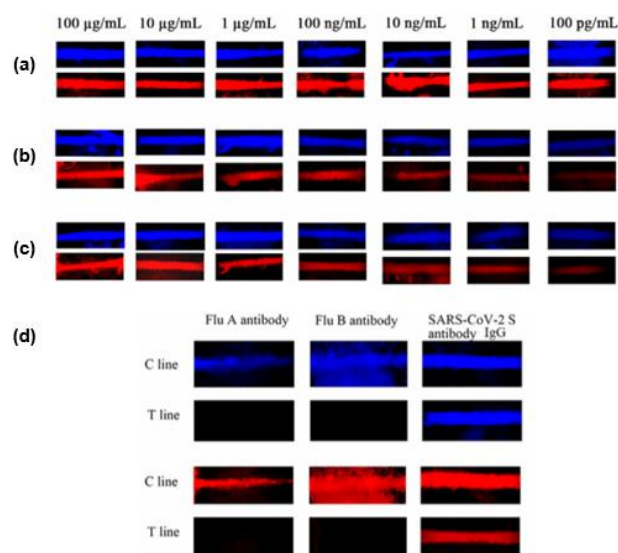


Figure 15. Simultaneous detection of IgG and IgM antibodies in a concentration range of 100 pg/mL to 100 µg/mL employing an LFIA based on AIE-CDs-SSP with the aid of fluorescence microscopy upon blue and green light irradiation. (a) C-line, (b) antibody IgM, and (c) antibody IgG. (d) Selectivity study of the AIE-CDs-SSP LFIA strip at 100 ng/mL of various antibodies using fluorescence microscope under blue and green light irradiation (adopted with permission by [10]).

For instance, Gamboa et al. [85] investigated the role of CDs for the electrochemical detection of biomarker, D-dimer (DD). Increased levels of DD in blood are associated with severe infection of COVID-19, as well as with other conditions, such as thrombosis. In this case, a screen-printed electrode (SPE) was functionalized with CDs and DD189cc monoclonal antibody in successive layers to enhance selectivity towards the antigen (Figure 16). The choice of this antibody relies on the increased need for specificity among the recognition of DD and other biological byproducts. The plentiful carboxyl and amine groups of CDs enabled the antibody's immobilization, while the BSA protein was used to block CDs' areas that were not covered with the antibody. CDs were synthesized based on a hydrothermal reaction of citric acid and urea. Electrochemical impedance spectroscopy (EIS) results revealed that CDs improved conductivity, electrode sensitivity, and electrochemical signal. The proposed biosensor exhibited competitive results in the determination of DD antigens in terms of the detection range (10–1000 ng/mL) and limit of detection (13.4 ng/mL) compared to other immunosensors. Moreover, the biosensor was applied for the determination of DD content in the plasma of a healthy patient with satisfactory recovery rates, indicating its potential to deal with complicated biological samples.

In another research, Martins et al. [86] proposed an electrochemical immunosensor for the detection of SARS-CoV-2 antibodies (IgG antibodies), modifying a screen-printed electrode (SPE) with GQDs and polyhydroxybutyrate (PHB). GQDs amplify electrochemical signals, whereas PHB contributes to the development of a core-shell structure with a

hydrophobic core and a shell with binding regions that allow interaction and binding with the RBD from the coronavirus spike protein (SARS-CoV-2). Particularly, GQDs were produced based on the pyrolysis of citric acid, and then cyclic voltammetry (CV) was employed for the electrodeposition of GQDs onto the surface of an SPE. The GQD-modified SPE was subsequently combined with PHB particles to facilitate the immobilization of the RBD of the SARS-CoV-2 spike protein. In the presence of IgG antibodies, a complex is formed as a result of the IgG antibodies' interaction with the RBD located at the electrode. This complex introduces both an insulating layer and steric hindrance, impairing the access of the redox probe to the electrode surface and resulting in a reduced faradaic response. The selectivity of the GQD-PHB-RBD immunosensor was evaluated using non-specific yellow fever (YF) antibodies as interferents and compared to the response toward anti-SARS-CoV-2 spike antibodies, using differential pulse voltammetry (DPV). As shown in Figure 17a, the YF antibodies produced negligible signal changes, whereas the existence of the target anti-spike antibodies led to a pronounced decrease in the electrochemical response, confirming specific RBD–antibody recognition. The selectivity assay was further performed to examine the ability of the proposed immunosensor to discriminate COVID-19-positive and negative human serum samples. Results showed that positive samples induced a clear decrease in the electrochemical signal, contrary to negligible changes observed for the signal of negative samples (Figure 17b). Out of eight samples tested, only one false positive and no false negatives were observed, confirming the sensor's high specificity and minimal matrix interference (Figure 17c).

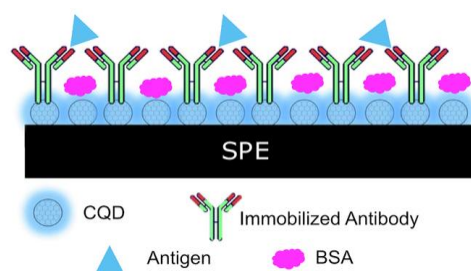


Figure 16. Schematic illustration of an immunosensor development based on CDs for the electrochemical detection of DD antigen (adopted with permission by [85]).

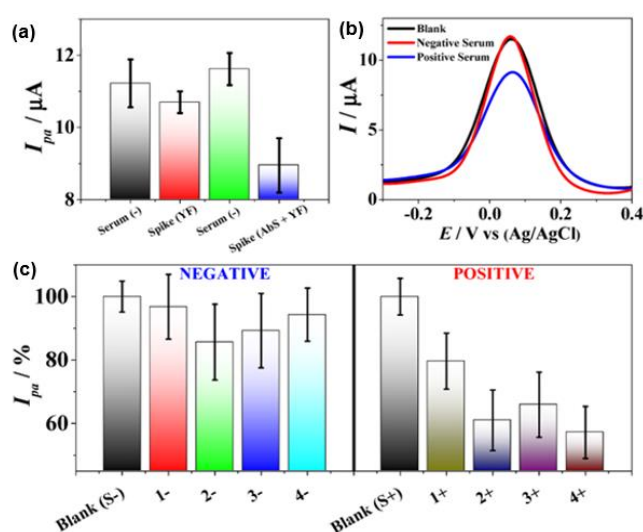


Figure 17. (a) Selectivity studies of GQD-PHB-RBD immunosensor on negative serum samples against YF antibodies, employing DPV. (b) DPV analysis for the determination of positive and negative serum samples. (c) DPV analysis for negative and positive human serum assays (adopted with permission by [86]).

5.4. Comparative Analysis of Different Detection Approaches

Following the discussion of individual studies, the application of CDs in COVID-19 detection can be categorized in three main areas according to the applied sensing principle: fluorescence methods, LFIA-based biosensors, and electrochemical methods. Several targets related to COVID-19 were evaluated as potential analytes for the examined sensors. For instance, the SARS-CoV-2 RNA N protein and S protein have been the target in many cases [80,82], whereas other studies are focused on the determination of COVID-19 antibodies, such as IgG and IgM [10,74,83,86]. Some studies report the detection of COVID-19-related molecules, such as D-dimer [85], the cytokine interleukin-6 [79], and neopterin [75]. Although the reported biosensors target different analytes, their analytical performance is affected not only by the sensing principle, but also by the physicochemical characteristics and synthesis of CDs. Table 1 summarizes the application of CDs for the detection of COVID-19, along with crucial parameters, such as analyte, limit of detection (LOD), linear range, recovery rates, and detection time.

Table 1. Summary of studies investigating the role of CDs in COVID-19 detection.

Biosensor	Target	Method	LOD	Linear Range	Recovery Rates	Detection Time	Ref.
Fe-S,N-co-doped CDs	Neopterin	FL	3.11 nM	0–10 nM	96–104%	-	[75]
CDs/antisense oligonucleotide (ASO) sequences	SARS-CoV-2 nucleocapsid (N) gene	FL	81 copies/ μ L	-	-	-	[77]
CDs	Molnupiravir	FL	800 ng/mL	2.500–70.000 ng/mL	99.53–101.79%	-	[78]
N-CDs/Au NPs/aptamer	interleukin-6 (IL-6)	FL	8.2×10^{-4} ng/mL	$(15-59) \times 10^{-4}$ ng/mL	95.7–102.9%	-	[79]
⁺ Au NPs/CDs-Ab	S protein	FL	0.05 ng/mL	10^{-4} –100 ng/mL	110.05–115.45%	12 min	[82]
Ag@Au NPs/GQDs	2019-nCoV mAb	FL	5×10^{-5} ng/mL	10^{-4} –10 ng/mL	90.8–103.3%	-	[81]
N, B-CDs	IgG and IgM	FL	-	-	-	-	[83]
CDs@SiO ₂ /Au NPs	N protein	FL	0.002 ng/mL	0.01–5 ng/mL	102.5–106%	-	[80]
CDs/RSCs	SARS-CoV-2 NP anti-gen	LFIA	0.1 ng/mL (UV light) 0.01 ng/mL (FL microscopy)	0.01–1.000 ng/mL	96.97–102.8%	-	[84]
SiO ₂ @Au@CDs	IgG	LFIA	1.2×10^{-4} ng/mL	7.4×10^{-4} –0.74 ng/mL	-	<15 min	[74]
AIE-CDs/SARS-CoV-2 spike protein	IgG and IgM	LFIA	0.1 ng/mL	0.1–100 $\times 10^3$ ng/mL	-	-	[10]
CDs/Ab-SPE	D-dimer	EC	13.4 ng/mL	10–1.000 ng/mL	60–70%	-	[85]
GQDs/PHB-SPE	Anti-S (IgG)	EC	100 ng/mL	100–10 $\times 10^3$ ng/mL	-	120 min	[86]

Abbreviation: LOD: limit of detection, FL: fluorescence, LFIA: lateral flow immunoassay, EC: electrochemical.

Fluorescence-based sensing of COVID-19 with CDs is the predominant diagnostic strategy owing to the intrinsic PL, aqueous solubility, and facile functionalization of CDs. Comparison of the reported studies highlights that fluorescent-sensing mechanisms can be implemented through fluorescence quenching, fluorescence recovery, FRET, aggregation-induced emission, and ratiometric fluorescence. Despite the fact that the above methods differ in their sensing implementation, they demonstrate the versatility of CDs as optical biosensors based on specific interactions with analytes. Among them, FRET constitutes a powerful and adaptable fluorescence-based strategy [79–82]. FRET relies on precise modulation of the donor–acceptor distance at the nanoscale, which is directly translated into

measurable fluorescence changes upon target recognition. The incorporation of biological recognition elements, including aptamers or antibodies, enables selective binding of viral antigens, cytokines, or antibodies. This results in changes in the spatial arrangement of the donor–acceptor pair and either suppresses or restores the FRET process.

Fluorescent LFIA represent an important evolution of conventional fluorescence biosensors by integrating CDs into portable POC testing platforms. CD-based LFIA platforms exhibit enhanced signal intensity, low detection limits, rapid analysis, and minimal user intervention. These features make LFIA platforms promising for decentralized testing and large-scale screening applications.

While fluorescence-based sensing strategies dominate in the application of CDs as diagnostic tools for COVID-19, electrochemical techniques have also emerged as a complementary and equally promising alternative [85,86]. Research studies have highlighted the beneficial role of CDs in the modification of commonly used electrodes, acting as electrochemical signals amplifiers; in combination with immunorecognition elements, CDs enable the development of electrochemical immunosensors with enhanced selectivity, sensitivity, and analytical performance. However, it is important to address the fact that the reported electrochemical biosensors for COVID-19 based on CDs remain relatively limited, and their analytical performance strongly depends on electrode fabrication, surface modification procedures, and measurement conditions, preventing meaningful comparison among the studies. Also, the currently available literature is insufficient to establish whether electrochemical platforms can consistently outperform fluorescence-based approaches. Additional studies involving different biomarkers and standardized analytical protocols are therefore required in order to establish meaningful conclusions regarding their analytical performance.

It is important to note that each sensing platform provides distinct advantages depending on the intended target rather than exhibiting universal superiority. Therefore, direct comparison among the proposed platforms remains challenging because different investigations target different analytes using distinct sensing mechanisms, biological matrices, calibration protocols, and performance metrics.

Apart from the sensing principle, the overall detection ability of CD-based biosensors is based on critical parameters, including the synthesis method, precursor molecules, functionalization, and post-synthesis modification. In particular, bottom-up approaches, such as hydrothermal or solvothermal treatment and microwave-assisted pyrolysis, are predominantly employed for the production of CDs used in the detection platforms. The choice of precursors is also vital for the resulting surface chemistry and optical properties of CDs. For instance, the use of molecular precursors such as citric acid, urea [85,86], EDA [79], thiourea [10], and EDTA [78], as well as biomass-derived sources (e.g., eggshells, plant leaves, or food waste) [78,80], directly determines the surface functional groups, elemental composition, and electronic structure of the resulting CDs. In this context, heteroatom doping (e.g., N, S, B, Fe, or co-doping elements) modulates the electronic structure, improves fluorescence properties, and introduces specific binding sites. Additionally, post-synthetic approaches, including carbodiimide coupling [10], metal–ion coordination [75], or bioconjugation with antibodies, aptamers, or oligonucleotides [77,79,82], play a decisive role in imparting selectivity and specific recognition of viral components. In parallel, purification steps such as dialysis are crucial for the selectivity of the as-proposed sensors [77]. All these underline that the rational design of CDs is essential for the fabrication of CD-based biosensors with increased sensitivity and specificity towards COVID-19.

Comparative analysis of the reported studies for COVID-19 detection based on CDs reveals that CD-based biosensors could offer practical advantages such as shorter analysis time, simplified operation, and reduced instrumentation requirements. For example, the

CD-based fluorometric assay (linear range: 2.5–70 µg/mL, LOD: 0.8 µg/mL) for molnupiravir determination exhibited analytical performance comparable to HPLC (linear range: 0.10–60.0 µg/mL, LOD: 0.05 µg/mL) and LC-MS/MS (linear range: 0.20–10.0 µg/mL) methods while avoiding expensive instrumentation and solvents waste [78]. Similarly, FRET-based biosensors with CDs achieved a favorable balance between sensitivity (LOD 0.05 ng/mL) and rapid analysis (12 min), whereas previously reported antigen detection platforms, including fiber optics and LFIA, resulted in sensitivities of 0.1 ng/mL and 0.033 ng/mL, respectively, and analysis of 20 min and 30 min, respectively [82]. Ratiometric fluorescence systems exhibited approximately 54-fold higher sensitivity compared to CD-based immunoassays [80]. CD-based LFIA strips exhibited excellent analytical performance with a broad working range (7.4×10^{-4} –0.74 ng/mL), low LOD (1.2×10^{-4} ng/mL), and short detection time (15 min) compared with previously reported LFIA platforms employing semiconductor quantum dots [74]. Since LFIA constitutes the sensing principle used in most rapid antigen tests, these findings highlight the potential of CD-based fluorescent LFIA platforms to provide rapid POC detection with enhanced analytical performance.

Despite the promising analytical performance of CD-based biosensors for COVID-19 detection, several limitations hinder their clinical translation. The majority of the presented studies are limited to laboratory validation using spiked serum samples instead of implementing large cohorts of clinical specimens. This implies the necessity of comprehensive assessment prior for their analytical performance under real clinical conditions. Another important limitation concerns reproducibility and standardization. Differences in synthesis conditions, precursor composition, heteroatom doping, purification procedures, and surface functionalization result in CDs with varying physicochemical characteristics. This indicates difficulties in batch-to-batch reproducibility and interlaboratory comparison. Finally, most of the studies underline the superb analytical sensitivity under laboratory conditions, but limited attention has been devoted to long-term storage stability, large-scale manufacturing, quality control, regulatory approval, and integration into commercially viable diagnostic devices. These issues represent major obstacles for the successful translation of CD-based biosensors from proof-of-concept laboratory studies to routine clinical practice.

6. Application of Carbon Dots as Antiviral Nanomaterials for COVID-19 Treatment

Viruses, including SARS-CoV-2, continuously evolve over time, and virus mutations can influence their transmissibility, spread, and the effectiveness of vaccines and treatment approaches. In the case of SARS-CoV-2, the accumulation of genetic alterations has led to the emergence of multiple variants, including Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2), which pose increased risks due to their enhanced transmissibility and severity of infection. The rapid emergence of these variants throughout the pandemic highlighted the urgent need for alternative and complementary antiviral strategies. In this context, nanotechnology-based approaches, designed to inhibit viral entry, replication, or propagation while maintaining low cytotoxicity, have attracted substantial interest. Among them, CDs have emerged as promising therapeutic agents in the treatment of viruses due to their unique physicochemical characteristics, such as their small size and tunable surface chemistry, which play a significant role in their antiviral performance [1,54].

To begin with, viral infection typically proceeds through four key stages: i. attachment, ii. penetration, iii. replication, and iv. budding. CDs can exert their antiviral effects through distinct mechanisms at various stages of the viral life cycle. The virus particles are initially attached onto the host cells prior to their penetration. CDs, exhibiting rich surface functionality, can bind to the virus membrane and alter the viral surface proteins, thereby impairing cellular physiology and ultimately hampering infection. Virus inactivation could

be the result of electrostatic interactions among positively charged CDs and negatively charged virus membrane, resulting in subsequent viral aggregation. The entrance of virus to the host cells can be inhibited from the CDs by changing the cell surface membrane and attached proteins. Since the virus has entered the host cells, one approach for virus inhibition includes the ceasing of replication. To achieve this, CDs are able to alter the required enzymes for viral genome replication or trigger the activation of interferon. Following replication, newly formed virions are released from the host cell through budding. Some antiviral strategies interfere with this release process and effectively limit viral spread and reduce infection severity. In some types of viral infections, excessive production of ROS is observed, which can further trigger DNA damage via apoptosis-related signaling pathways. CDs have been also used in these cases to mitigate virus-associated oxidative stress. Despite the fact that the above mechanisms describe the main antiviral mechanisms of CDs, some research studies present the antiviral performance of CDs without clarifying the precise mode of action. This intensifies the necessity of exploring all the possible pathways for the role of CDs in viral inactivation or suppression [2,11,57].

Recent studies have explored the utilization of CDs in COVID-19 treatment, as they combine unique physicochemical properties with high biocompatibility, along with ease-of-preparation, low-cost efficiency, and abundance of precursors, including biomass, thereby enhancing the overall sustainability of these nanomaterials. The following section critically discusses representative studies reporting the therapeutic potential of CDs against SARS-CoV-2. Particular emphasis is placed on their ability to inhibit viral entry, interfere with viral replication, and serve as vaccine nanoplatforms. Simultaneously, the effect of precursor selection, surface chemistry, and particle size of CDs on their antiviral performance is highlighted.

6.1. Carbon Dots Targeting Viral Entry

The inhibition of viral entry into host cells represents one of the most promising antiviral mechanisms of CDs against SARS-CoV-2. More specifically, CDs could interact with viral proteins or/and host cell receptors, thereby interfering with the virus–host recognition process and limiting viral attachment and entry into susceptible cells. Recent studies have demonstrated that the antiviral activity of CDs is influenced by several parameters, including the precursor materials, the surface functional groups, and the size of CDs.

To begin with, the research study of Saikia et al. [87] presented an alternative low-cost carbon precursor for the synthesis of CDs with antiviral properties. In particular, CDs were synthesized via the ultrasonication of coal in the presence of hydrogen peroxide. The use of coal resulted in CDs with smaller size, improved aqueous solubility, and biocompatibility. Furthermore, coal-derived CDs possess abundant surface functional groups (-OH, -COOH) that facilitate interaction with the viral surface. In vitro studies showed no cytotoxicity in Vero E6 and HEK-293 cell lines after 24 h incubation with coal-derived CDs at concentrations of 100 µg/mL and 200 µg/mL, respectively (Figure 18a,b). To assess the potent antiviral performance of CDs, Vero cells were then infected with SARS-CoV-2, and a dose-dependent inhibitory effect was observed. Coal-derived CDs achieved 99.3% viral inhibition at 100 µg/mL with an IC₅₀ (inhibitory concentration 50%) of 5.469 µg/mL. Subsequently, qRT-PCR analysis estimated a Ct value of 28.350 for CDs, comparable to the FDA-approved antiviral drug Remdesivir (29.313). Based on DCFH-DA staining in HEK-293 cells, the antiviral activity of coal-derived CDs does not involve a ROS-mediated mechanism, as no ROS generation was detected (Figure 18c). A possible explanation for the SARS-CoV-2 inhibitory action of coal-derived CDs may rely on the binding of CDs to the SARS-CoV-2 spike protein. Specifically, the abundant hydrophilic

surface functional groups of CDs may bind to the SARS-CoV-2 spike protein, thereby interfering with virus–host cell recognition and entry. Alternatively, CDs possibly interact with nucleocapsid proteins, suppressing viral replication and budding processes.

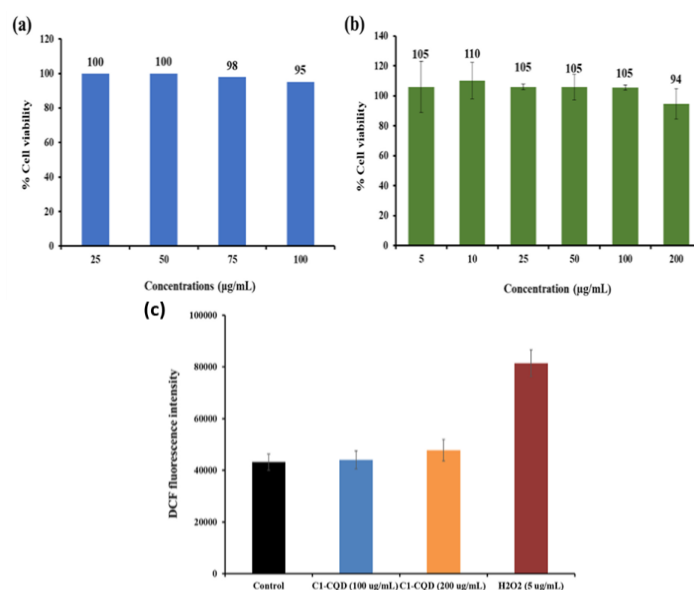


Figure 18. Coal-derived CDs as antiviral agents. In vitro studies in (a) Vero E6 and (b) HEK-293 for various concentrations of CDs. (c) ROS production studies of coal-derived CDs (C1-CQD) at different concentrations in HEK-293 cells (adopted with permission by [87]).

Biomass-derived CDs have also emerged as a promising class of antiviral agents, not only because of their low toxicity and favorable biocompatibility but also due to environmental sustainability and eco-friendly synthesis routes. In this respect, Nair et al. [88] utilized *Withania somnifera* (L.), a plant known as Indian winter cherry or Indian ginseng, which belongs to the family of Solanaceae, and it is cultivated in subtropical and tropical zones as a precursor for CD synthesis. Initially, the dried raw material was dispersed into ultra-pure water and then transferred into an autoclave at 180 °C for 12 h (Figure 19). The same procedure was repeated twice with the addition of ammonia (NH₃) and then ethylenediamine (EDA), respectively, to act as N-doping agents. Therefore, three samples (CDs, NH₃-CDs, EDA-CDs) were obtained and further evaluated. Both N-doped CD samples exhibited a red-shifted PL emission at 380 nm compared to the pristine CDs, which emitted at 320 nm, confirming the crucial role of CD doping on their optical properties. The fluorescence quantum yield of CDs, NH₃-CDs, and EDA-CDs was 5.6%, 32.5%, and 27.6%, respectively, indicating that the introduction of N through NH₃ and EDA creates additional electronic states that reduce non-radiative recombination pathways, thereby enhancing the quantum yield. All CDs (diluted at a ratio of 1:1000) were non-toxic to DLD1 cells (ACE2-expressing). Regarding the antiviral study, a pseudovirus was used to carry the SARS-CoV-2 spike protein and a red fluorescent marker (TdTomato). In the control groups, approximately 20% of cells exhibited fluorescence, corresponding to the normal infection efficiency of the S-protein. When the cells were exposed to pseudovirus, CDs, NH₃-CDs, and EDA-CDs samples (1:1000 diluted) at 3%, 4%, and 5% displayed fluorescence, indicating 85%, 80%, and 75% inhibition, as shown in Figure 20. According to the proposed mechanism of action of CDs against SARS-CoV-2, their antiviral effects are attributed to the interactions occurred among their surface functional groups, such as hydroxyl, carboxyl, and nitrogen-containing moieties introduced via NH₃ and EDA addition, as well as the viral S-protein. These interactions are suggested to interfere with spike–ACE2 binding, leading to enhanced blocking of viral entry. Nevertheless, the conduction of clinical assessments

is crucial to evaluate the efficiency of CDs in SARS-CoV-2 treatment. In a related study, presented by Kalkal et al. [12], it is proposed that CDs derived by *Allium sativum* (garlic) could serve as a novel nanomedicine platform for the COVID-19 theranostic. The authors suggest that the intrinsic immunomodulatory, antioxidant, and antimicrobial/antiviral properties of garlic, combined with the fluorescent characteristics of CDs, may enable the development of an agent capable of simultaneous diagnosis and therapy.

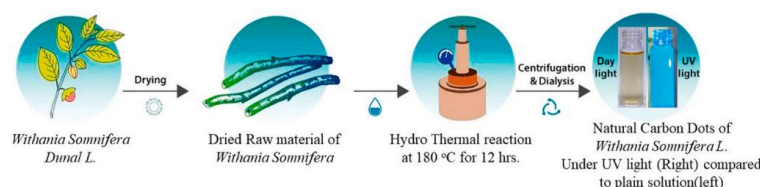


Figure 19. Biomass-derived CDs obtained through a facile and eco-friendly hydrothermal treatment of *Withania somnifera* (L.), a plant that is cultivated in subtropical and tropical climates (adopted with permission by [88]).

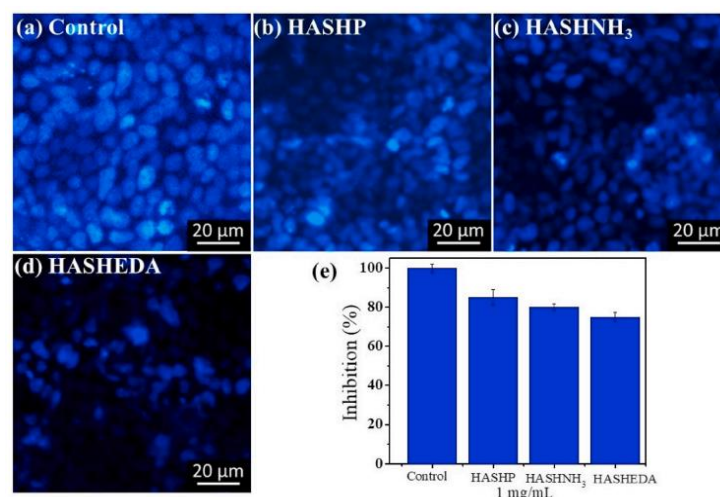


Figure 20. Fluorescence microscopy images of fluorescent DLD1 cells (expressing human ACE2) under (a) control conditions and after treatment with (b) CD (HASHP), (c) NH₃-CD (HASHNH₃), and (d) EDA-CD (HASHEDA) samples. (e) Bar diagram of inhibition (%) of (a–d) (adopted with permission by [88]).

There is no doubt that surface chemistry plays a significant role in dictating not only excellent aqueous solubility and biocompatibility, but also their ability to recognize and bind viral components. Consequently, numerous studies have investigated the effect of CD functionalization on their physicochemical and antiviral properties. For example, Hsieh et al. [89] synthesized three distinct GQDs samples, with different distributions of oxygen- and nitrogen-containing functional groups, highlighting how surface chemistry influences the interactions with the RBD of the spike (S) protein. Briefly, GQDs-1, GQDs-2, and GQDs-3 were prepared by a solid-phase microwave-assisted technique, using different weight ratios of citric acid:urea (1:3, 1:1, 3:1). As confirmed by ICP-OES/MS and XPS analyses, all samples were highly hydrophilic (O/C ratios = 56.2–65.0 at.%), while the N/C atomic ratio was increased in proportion to the urea content. Herein, it is important to note that all GQD samples possess a high amidation level, where the pyrrolic or pyridinic N (C=N–C) and quaternary N (N–C₃) (Figure 21a) are the major contributors; however, only the sample GQDs-3 contains amide–carbonyl groups (O=C–NH₂) groups, located at the edges of the graphitic sheets. The toxicity profile of GQDs was systematically assessed in H171 and H184 human cells. Cell viability of H171 cells remained over 80% for all samples when incubated for 72 h at a 0.25–50 µg/mL concentration range. Additional assays in H184 cells

exposed to GQDs-3 (100 µg/mL, 72 h) confirmed adequate cell viability. The as-synthesized GQDs samples were employed as inhibitors to combat *Feline Coronavirus* NTU156 (FCoV NTU156), which belongs to the *Coronaviridae* family, and it is a positive-sense, single-stranded RNA virus with a lipid envelope. As it was observed, the addition of GQDs-1 and GQDs-2 after 5 min of infection with the virus presented an inhibition of 28.87%. On the other hand, GQDs-3 achieved >90% inhibition against FCoV NTU156, indicating the critical role of surface chemistry in nanoparticle antiviral activity. Notably, HR-TEM was performed to elucidate the antiviral mechanism, as shown in Figure 21b,c. Herein, the antiviral mechanism of functionalized GQDs could be classified as a multimodal process with predominant viral entry inhibition. HR-TEM results show strong adsorption of GQDs onto the virus surface, driven by interactions between surface functional groups and the spike protein. This adsorption blocks early-stage infection by hindering spike-receptor binding, thereby preventing viral entry into host cells. In addition, GQDs exhibit secondary antiviral effects through light-induced ROS generation and membrane disruption, leading to oxidative damage of viral proteins and RNA. Furthermore, interactions of phenolic and amide–carbonyl functional groups with the viral lipid envelope promote membrane disruption and structural collapse of the virus. Overall, the antiviral activity arises from a combination of entry inhibition, ROS-mediated inactivation, and membrane-disruptive virucidal effects, with entry blocking being the dominant pathway.

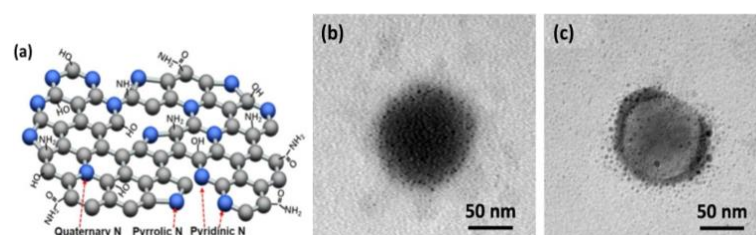


Figure 21. (a) Schematic representation of N-functionalized GQDs structure, prepared by a solid-phase microwave-assisted technique. HR-TEM images of (b) GQDs-adsorbed onto FCoV NTU156 and (c) GQDs penetration through the virus, leading to lipid membrane disruption (adopted with permission by [89]).

Similarly, Ferreira et al. [68] investigated the correlation between thiol functionalities of CDs and SARS-CoV-2. CDs derived from thiol compounds could theoretically contain increased number of sulfhydryl (-SH) groups compared to thiol drugs (e.g., N-acetylcysteine (NAC)) due to their small size (<10 nm) and high surface-to-volume ratio. The available thiol-based drugs present up to 2 mol of thiols per mol of drug, while their clinical application may be limited due to toxicity or reduced therapeutic efficacy. Thus, CDs have been considered a promising substitute. In their study, CDs were prepared via a microwave technique, using cysteamine and citric acid as precursors in molar ratios of 1:1 (CystaD 1-1) and 2:1 (CystaD 2-1) so as to evaluate the effect of thiols on their antiviral activity. The concentration of -SH groups was determined with Ellman's Reagent and quantified by Beer-Lambert's Law, based on adsorption measurements using a UV-Vis spectrophotometer. Based on the results, the CystaD 2-1 sample possesses approximately 3.5 times higher number of thiols than the CystaD 1-1 sample. Herein, it is worth mentioning that thiol-based drugs mitigate COVID-19 both by disrupting spike disulfide bonds and by scavenging ROS. Therefore, the authors performed a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging assay to evaluate the antioxidant potential of CDs. The samples showed a significant concentration-dependent decrease in DPPH (Figure 22a), with CystaD 2-1 reducing DPPH more than CystaD 1-1 in all cases. Given the central role of oxidative stress in driving inflammatory responses during viral infection, the potential of CDs to modulate intracellular ROS was evaluated. DHE fluorescence measurements

showed that CystaD 2-1 at 0.8 mg/mL produced a significant reduction in cellular ROS and even outperformed the antioxidant control NAC (Figure 22b). The observed ROS reduction indicates that CystaD 2-1 is a promising anti-inflammatory agent with potential for symptom relief of COVID-19. The cytotoxicity of thiol-containing CDs was tested in a concentration range of 0.05–0.80 mg/mL using monkey Vero E6 kidney and human embryo kidney HEK 239T cell lines via the MTS assay. Over 90% of cells survived at all of the tested concentrations for both CystaD 1-1 and CystaD 2-1 in Vero E6, as well as HEK 293T. Thiol-functionalized CDs were further tested in a viral entry inhibition assay using VSV-SARS-CoV-2 spike pseudo-typed particles on Vero E6 cells. As it is observed in Figure 22c, CDs exhibit significantly low viral entry levels. CDs, which contain higher amounts of -SH groups (CystaD 2-1), present increased viral inhibition. Therefore, the results of this study pave the way for the utilization of thiolated CDs in the treatment of COVID-19 and other coronavirus-related diseases. To summarize, the antiviral activity of thiol-functionalized CDs is primarily driven by inhibition of SARS-CoV-2 entry through binding to the spike RBD, combined with thiol-mediated disruption of disulfide bonds that destabilizes spike structure, while additional antioxidant activity contributes to modulation of virus-induced oxidative stress. Ramezani et al. [90] have also studied the interactions among various functional groups of CDs with the S protein of SARS-CoV-2 through molecular docking and molecular dynamics. Briefly, the results showed that OH- and NH₂-OH-functionalized CDs can effectively block RBD with 80% and 85% site occupancy and the most favorable binding energies (−699.3 kJ/mol and −689.7 kJ/mol, respectively). Consequently, CDs may disrupt the interaction between the viral spike protein and the host receptor, as suggested by the *in silico* simulations.

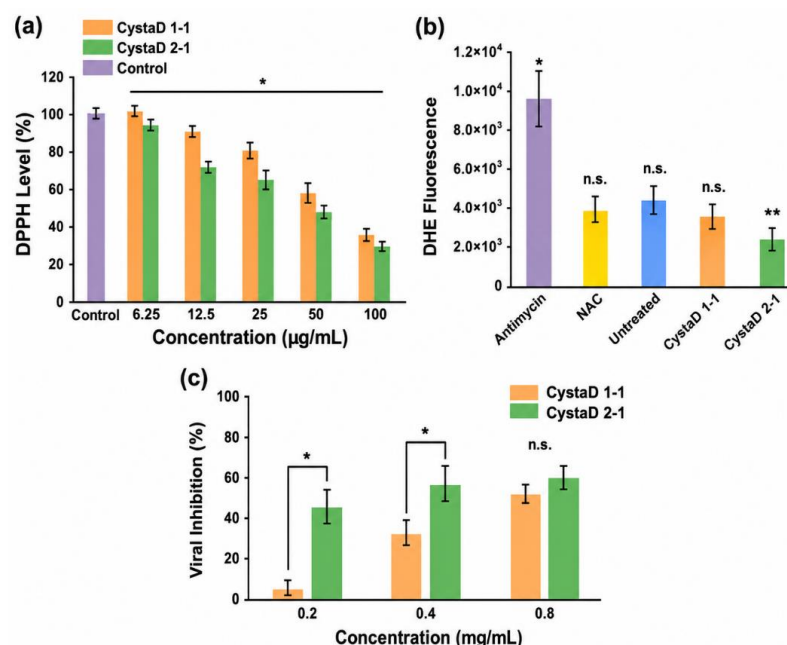


Figure 22. (a) Free-radical scavenging assay showing the consumption of DPPH by thiol-based CDs, containing different amounts of -SH groups. (b) DHE assay comparing CDs (0.8 mg/mL) with control samples. (c) Viral inhibition of both CDs. * and ** = statistical significance (SS) difference from untreated control (* $p < 0.05$, and ** $p < 0.01$) (adopted with permission by [68]).

According to a recent study [91], both surface chemistry and particle size are other important factors that may influence the antiviral activity of CDs. In this work, GQDs were prepared through acidic oxidation of carbon black, followed by dialysis for 3 days, using a dialysis bag of 3 kDa molecular weight. The filtered GQDs and those remaining in the bag were named as GQDs-S and GQDs-L, respectively. GQDs-S and GQDs-L present

different fluorescent properties, while their hydrodynamic sizes were 2.58 ± 0.99 nm and 5.89 ± 1.88 nm, respectively. Furthermore, based on FT-IR analysis, the C/O ratio of GQDs-S was decreased compared to GQDs-L, indicating their enhanced hydrophilicity. Both samples showed no toxicity on Madin-Darby canine kidney (MDCK) cells at 5 mg/mL. HEK293T cells expressing human ACE2 and TMPRSS2 (293T-ACE2/TMPRSS2) were used to evaluate inhibition of SARS-CoV-2 (72 h infection). Interestingly, at a concentration of 0.0625 mg/mL, GQDs-S failed to inhibit SARS-CoV-2. On the other hand, GQDs-L suppressed viral infection with $IC_{50} = 0.013$ mg/mL. The enhanced antiviral performance of GQDs-L appears to arise from the combined influence of particle size and surface chemistry. The proposed antiviral mechanism of GQDs-L was attributed mainly to their ability to interact with the viral envelope. The increased hydrophobicity and amphiphilic character of GQDs-L promoted stronger interactions with the viral lipid envelope, leading to direct virucidal membrane disruption, structural damage, and subsequent loss of viral infectivity. Thus, GQDs-L appears to act predominantly at the early stages of infection by impairing viral attachment and entry into host cells. GQDs-S and GQDs-L were also tested against other viruses, such as influenza A, where opposite antiviral effects were observed, likely due to differences in viral envelope membrane composition. These findings highlight that the antiviral efficacy of GQDs is governed by particle size and surface chemistry, as well as the lipid characteristics of the target virus.

6.2. Carbon Dots Targeting Viral Replication

While many studies focus on the ability of CDs to inhibit viral entry, increasing evidence suggests that they could also target post-entry stages of infection. In particular, through interactions with viral RNA, replication-associated enzymes, or host antiviral signaling pathways, CDs can suppress viral genome amplification and reduce the production of infectious progeny.

Taking into account the excellent antiviral properties of porphyrin against several viruses, including HIV and influenza A virus, researchers employed porphyrin as a precursor compound for the synthesis of CDs with enhanced antiviral activity [92]. Porphyrin is an organic molecule known for its distinctive optical and biological properties. However, the limited water solubility of porphyrin restricts its clinical application. In this case, CDs were fabricated based on the solvothermal treatment of porphyrin, citric acid, and urea. The proposed antiviral agent was evaluated with the aid of a dimer-based screening system (DBSS), a screening platform designed to target the SARS-CoV-2 nucleocapsid protein at its C-terminal domain (N-CTD). Porphyrin-derived CDs demonstrated improved ability to inhibit the dimerization of the SARS-CoV-2 N-CTD, compared to porphyrin itself. This increased bioavailability is primarily attributed to the improved aqueous solubility of porphyrin. Additionally, porphyrin-derived CDs showed lower toxicity towards Vero E6 cells than porphyrin, with CC_{50} (50% cytotoxicity concentration) values of 55.7 μ g/mL and 15.5 μ g/mL, respectively. Further studies on the viability of SARS-CoV-2 infected cells revealed increased performance for the porphyrin-derived CDs, with a strong antiviral effect observed at 4 μ g/mL, compared to 6 μ g/mL required for porphyrin. Moreover, the antiviral activity of CDs was assessed in terms of the maximum viral load they could effectively inhibit. Figure 23 illustrates the cytopathic effect reduction (CPE) on SARS-CoV-2-infected Vero E6 cells with several viral loads upon incubation with CDs. For SARS-CoV-2 infection up to 4000 plaque-forming units (PFUs), CDs achieved CPE more than 30%, whereas only 8.4% CPE was recorded for a viral load of 8000 PFU. The antiviral activity of porphyrin-derived CDs lies in their ability to target the C-terminal domain of the SARS-CoV-2 nucleocapsid protein. Given the essential role of the nucleocapsid protein in viral RNA packaging and genome stabilization, the impairment of its dimer

formation is expected to adversely affect viral replication and particle assembly. The as-proposed mechanism was further supported by *in silico* analysis. More specifically, *in silico* analysis revealed that porphyrin could strongly bind to key residues located at the nucleocapsid dimerization interface, theoretically inhibiting homodimer formation. Taking into account the role of nucleocapsid in viral genome packing and replication, disruption of nucleocapsid function could impair viral propagation. In the case of porphyrin-derived CDs, enhanced antiviral activity and reduced cytotoxicity were recorded compared to free porphyrin, which can be attributed to surface chemistry, facilitating stronger interactions with the target viral protein. Nevertheless, while the DBSS assay provides experimental support for interference with N-CTD dimerization, further studies are required to fully validate the intracellular binding dynamics, as well as the proposed mechanistic antiviral pathway of CDs.

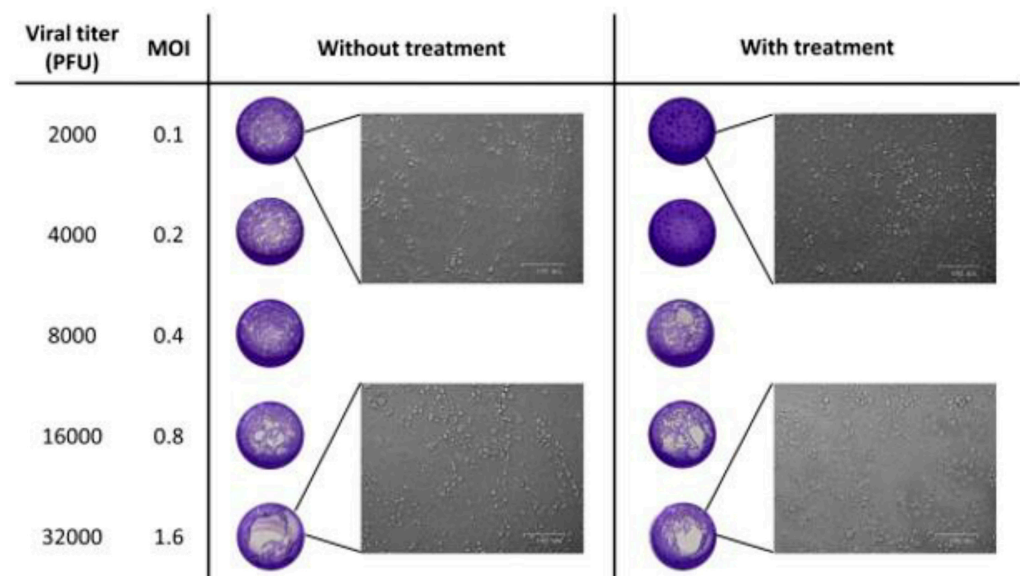


Figure 23. CPE reduction in SARS-CoV-2-infected Vero E6 cells across different viral loads without and with the treatment of 4 µg/mL porphyrin-derived CDs. Representative images of crystal violet-stained wells are provided, along with corresponding inverted-microscope observations highlighting CPEs in the cell monolayers (adopted with permission by [92]).

In a similar study, Fibriani et al. [93] synthesized curcumin-derived CDs as a potential antiviral drug against COVID-19. Curcumin, the main component of turmeric, is well known for its beneficial properties (e.g., anti-inflammatory, antioxidant, antimicrobial, anticancer, etc.). Although curcumin has been previously tested and presented antiviral activity against SARS-CoV-2, its clinical application is hindered due to its hydrophobic nature and low bioavailability. On the other hand, CDs synthesized using curcumin as a precursor material present increased solubility and bioavailability. More specifically, the curcumin-derived CDs were prepared via a solvothermal method; during this process citric acid, urea and curcumin were dispersed in ethanol, and the solution was transferred into an autoclave at 160 °C for 5 h. In this context, the potential of curcumin-derived CDs to inhibit the dimerization of the N-CTD was investigated using molecular docking, DBSS in *E. coli*, and SARS-CoV-2-infected Vero E6 cell-culture assays. The binding affinity and % interaction between SARS-CoV-2 N-CTD (PDB ID: 6ZCO) and the candidate compounds were investigated by *in silico* molecular docking, and the results are presented in Figure 24a. The study was carried out for curcumin and a series of hypothetical curcumin-derived CDs structures (amino, graphitic, pyridinic, and pyrrolic), considering both their keto and keto-enol tautomeric forms. As it was observed, all compounds had negative free-energy values,

meaning binding is predicted to be spontaneous. However, curcumin-derived CDs exhibit favorable binding (more negative ΔG) compared to curcumin, as well as higher engagement of dimerization-relevant residues, with an increased number of non-covalent π interactions at the N-CTD interface (Figure 24b). Consequently, molecular docking demonstrated that the CDs interact strongly with residues located at the N-CTD dimerization interface, exhibiting more favorable binding energies compared to curcumin and establishing multiple non-covalent π interactions. The aforementioned interactions are proposed to prevent or destabilize N-CTD homodimer formation, thereby inhibiting the assembly of functional nucleocapsid complexes required for efficient viral genome encapsidation. Screening of the drug candidates using the DBSS aligns with the above molecular docking results. Both curcumin and curcumin-derived CDs could inhibit N-CTD dimerization in a living-cell reporter system, with the latter presenting higher fluorescence, suggesting stronger inhibition. Furthermore, both agents were non-cytotoxic up to 10 $\mu\text{g/mL}$ in Vero E6 cells, with CC_{50} values of 30 $\mu\text{g/mL}$ for curcumin and 11.67 $\mu\text{g/mL}$ for CDs, indicating acceptable safety at antiviral activity evaluations. Antiviral assays were performed by separately culturing Vero E6 cells with treatment of the curcumin and CDs at different concentrations for 72 h after infection of SARS-CoV-2 at multiplicity of infection (MOI) = 0.1. As shown in Figure 25, curcumin increased cell viability to 83.3% with complete suppression of CPE at 6 $\mu\text{g/mL}$, whereas CDs achieved 100% viability and 0% CPE with only 4 $\mu\text{g/mL}$, providing evidence for the enhanced inhibitory capability of CDs structure toward N-CTD dimerization. Taken together, the molecular docking, DBSS, and Vero E6 cells-based antiviral assays suggest that curcumin-derived CDs suppress SARS-CoV-2 replication by interfering with N-CTD dimerization and the subsequent formation of functional nucleocapsid complexes. These findings highlight the potential of CDs as a promising candidate for the development of nucleocapsid-targeting antiviral nanotherapeutics.

6.3. Carbon Dots as Vaccine Nanoplatfoms

CDs have emerged as promising nanoplatfoms for vaccine delivery and immunotherapy applications, owing to their unique properties, such as their small size, high biocompatibility, and tunable surface chemistry, which enable the efficient loading and delivery of antigens, nucleic acids, and immunomodulatory agents. In vaccine formulations, CDs can enhance antigen stability, facilitate cellular uptake by antigen-presenting cells, and improve antigen presentation, thereby strengthening both humoral and cellular immune responses. Furthermore, they could act as adjuvant-like nanocarriers, promoting prolonged immune stimulation and generation of immunological memory. As a result, CDs have been considered as attractive candidates for the development of next-generation vaccines against infectious diseases, including SARS-CoV-2 and its emerging variants.

The Omicron variant of COVID-19 is a highly mutated strain of SARS-CoV-2 that was first identified in late 2021 and rapidly spread across the globe. It contains an unusually large number of mutations in the RBD of the spike protein, increasing its transmissibility and ability to partially evade immune protection from prior infection or vaccination. Its emergence highlighted the importance of next-generation vaccines that provide strong and broad protection against Omicron-included variants. On this basis, Lei et al. [94] developed an intranasal vaccine containing cationic crosslinked CDs and a SARS-CoV-2 antigen, RBD-HR, with spontaneous antigen partialization. In their study, CDs were synthesized via a solvothermal method using PEI as a carbon source in a mixture of ethanol and H_2O_2 . Following this, CDs were polymerized via a ring-opening reaction using a linker. The cationic crosslinked CDs (CCDs) could attract RBD-HR antigens with anionic polypeptides through electrostatic interactions (Figure 26a). Different mass ratios of CCDs:RBD-HR were tested, and it was found that for the 10:1 mass ratio, the maximal

antigen-carrying rate was achieved. CCDs with a positive surface charge interact with negatively charged antigens, such as RBD-HR, enabling the efficient antigen condensation and the formation of larger nanoparticles. More specifically, CCDs:RBD-HR (10:1) could form stable spherical nanoparticles (~60 nm), as observed in Figure 26b, that protect RBD-HR from enzymatic degradation in the nasal cavity during intranasal immunization. This enhanced stability is attributed not only to multivalent electrostatic interactions between the positively charged CCDs and the negatively charged antigen, but also to additional non-covalent interactions. This multivalent electrostatic binding not only stabilizes the antigen but also enhances mucosal adhesion and prolongs antigen retention at the intranasal administration site. In addition, hydrogen bonding and other non-covalent interactions between surface functional groups of CCDs and protein epitopes may further stabilize the antigen–CCD complexes without disrupting antigen integrity. The crosslinked carbon framework also contributes to structural rigidity and resistance to enzymatic degradation, thereby improving *in vivo* stability. Collectively, these physicochemical features govern the ability of CCDs to modulate antigen presentation and immune activation, highlighting the critical role of surface chemistry in determining the biological performance of CD-based vaccine nanoplatfroms. Animals, including mice and rabbits, were immunized at days 0, 14, and 28, and in both cases, it was observed that serum antigen-specific IgG titer was increased when RBD-HR was administered with CCDs, compared to RBD-HR alone, suggesting the induction of both T-helper types (Th-1 and Th-2). The production of serum antibodies blocked binding between RBD and ACE2 receptors and neutralized the authentic viruses and pseudoviruses of SARS-CoV-2 (Figure 26c). Additionally, the immunoglobulin A (IgA) antibodies production was also evaluated. As anticipated, robust anti-RBD-HR IgA responses were observed in both bronchoalveolar lavage (BAL) fluid and serum of mice vaccinated with CCDs/RBD-HR, but not in mice receiving RBD-HR or PBS, indicating the establishment of strong mucosal immunity. WT female BALB/c mice being sensitive to Omicron variant infection were vaccinated with PBS, CCDs, and CCDs/RBD-HR. The latter conferred complete protection against Omicron infection in the upper and lower respiratory system, as viral RNA in nasal turbinates, trachea, and lungs was almost undetectable. In addition, the authors demonstrated that the CCDs/RBD-HR elicited long-lasting and broad humoral immunity. RBD-HR specific serum IgG titers remained relatively high even 260 days after the prime immunization, whereas the day-260 CCDs/RBD-HR was still able to neutralize both ancestral and multiple variant pseudoviruses of SARS-CoV-2. Moreover, the vaccine also induced robust cellular and tissue-resident immunity, with increased frequencies of IFN- γ -producing CD4⁺ and CD8⁺ T cells in lung tissue and bronchoalveolar lavage, as well as a marked expansion of CD69⁺ and/or CD103⁺ tissue-resident memory T cells (T_{RM}) in the respiratory tract, which are considered critical for rapid local control of respiratory viral infections.

6.4. Comparative Analysis of Different Therapeutic Strategies

The aforementioned studies demonstrated the considerable potential of CD-based therapeutic strategies against COVID-19. Based on their primary therapeutic mechanism, these approaches could be classified into two main categories: (i) CDs as direct antiviral agents, targeting viral entry and viral replication, and (ii) CDs as vaccine nanoplatfroms. Table 2 summarizes the CD-based therapeutic approaches reported in the literature according to the above classification. Although these strategies showed promising antiviral efficiency, a deeper analysis reveals substantial differences regarding the targeted stage of the viral life cycle, the action mechanism, and the experimental validation. Herein, a critical comparison of these therapeutic approaches is presented, highlighting their mechanisms

of action, advantages, limitations, and the major challenges that currently hinder their clinical application.

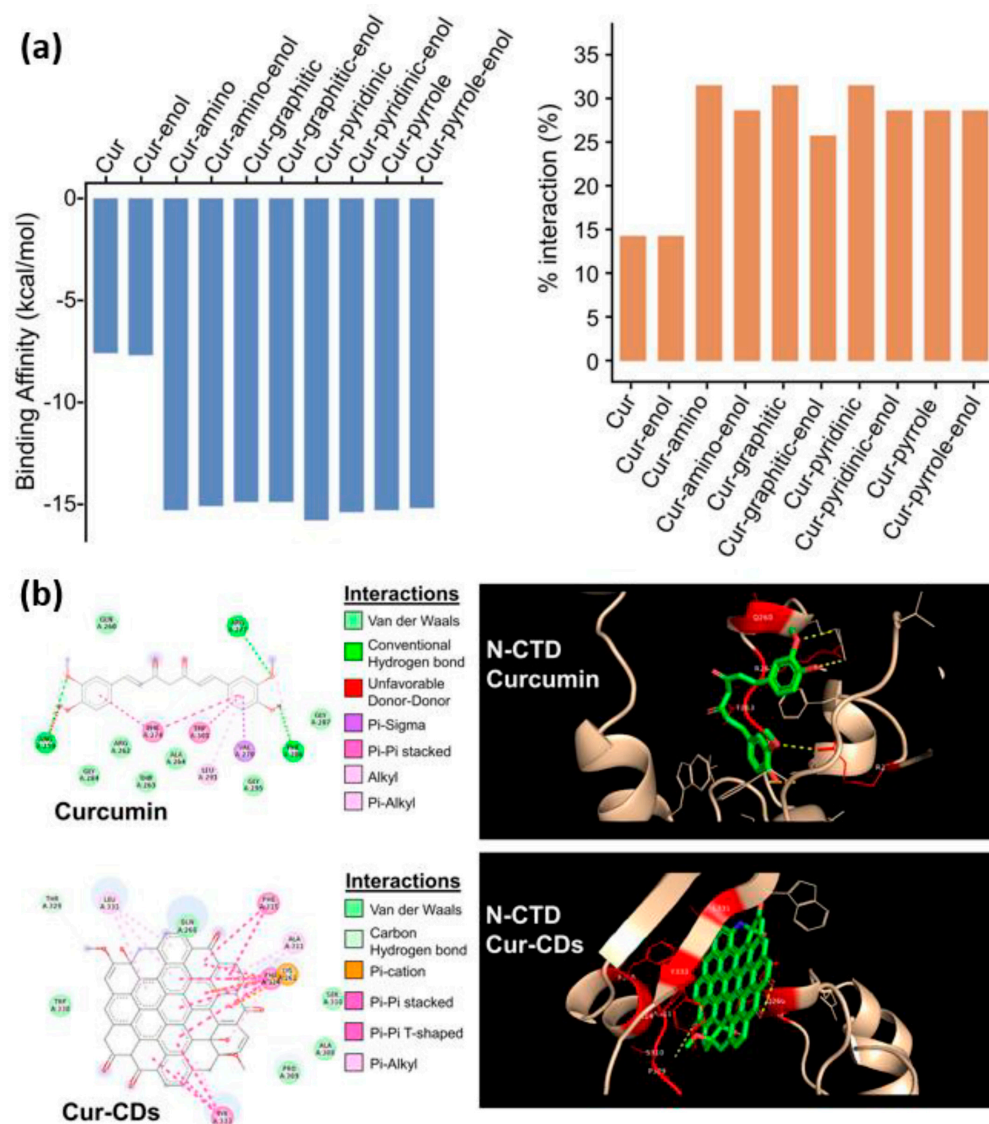


Figure 24. (a) Binding affinity (kcal/mol) and % interaction between SARS-CoV-2 N-CTD and curcumin and curcumin-derived CDs in both its keto and keto-enol tautomeric forms. (b) In addition, 2D and 3D visualization, where enhanced interactions among SARS-CoV-2 N-CTD and curcumin-derived CDs occurred (adopted with permission by [93]).

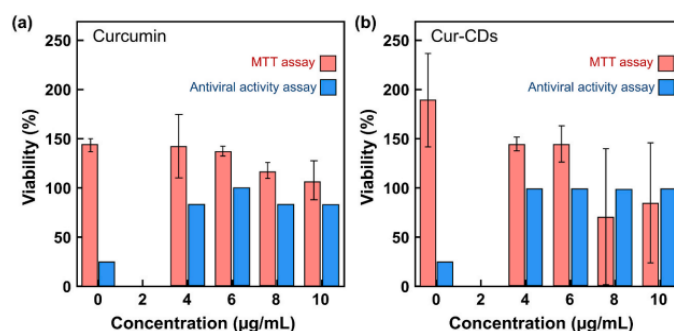


Figure 25. The viability of Vero E6 cells treated with (a) curcumin and (b) curcumin-derived CDs (adopted with permission by [93]).

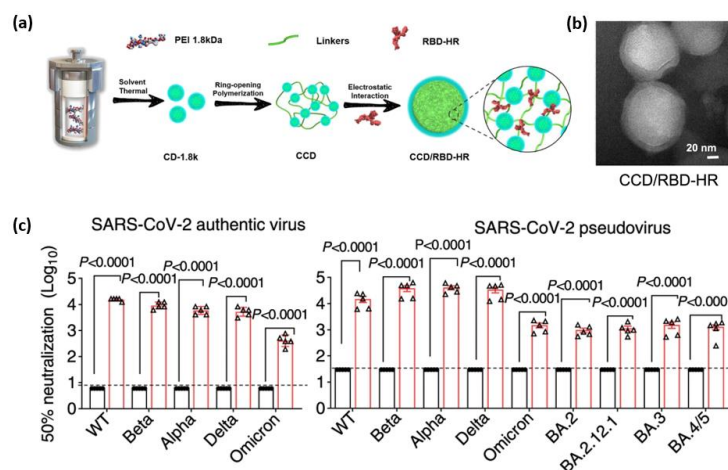


Figure 26. (a) Synthesis procedure of cationic crosslinked CDs. (b) TEM image of cationic crosslinked CDs/RBD-HR. (c) Neutralization of authentic viruses and pseudoviruses of SARS-CoV-2 (adopted with permission by [94]).

Table 2. Summary of the reported CD-based therapeutic strategies against COVID-19.

Carbon Dots	Target	Therapeutic Strategy	IC ₅₀	CC ₅₀	Viral Inhibition	Ref.
Coal-derived CDs	SARS-CoV-2	Viral Entry	5.469 µg/mL	95% cell viability	99.3% (100 µg/mL, 24 h)	[87]
<i>Withania somnifera</i> (L.)-derived CDs	Undoped CDs				85%	[88]
	N-doped CDs (NH ₃)	SARS-CoV-2 pseudovirus	-	-	80%	
	N-doped CDs (EDA)				75%	
Citric acid:Urea-derived N-doped GQDs	(1:3)	Feline Coronavirus NTU156	-	>80% cell viability	25.87% (10 mg/mL, 5 min)	[89]
	(1:1)				25.87% (10 mg/mL, 5 min)	
	(3:1)				>90% (10 mg/mL, 5 min)	
Thiol-functionalized CDs	VSV-SARS-CoV-2 pseudovirus	Viral Entry	-	-	<60.4%	[68]
GQDs	SARS-CoV-2 pseudovirus	Viral Entry	0.013 mg/mL	-	Completely inhibited (0.0625 mg/mL)	[91]
Porphyrin-derived CDs	SARS-CoV-2	Viral Replication	-	55.7 µg/mL	Strong dose-dependent inhibition of SARS-CoV-2 and N-CTD dimerization	[92]
Curcumin-derived CDs	SARS-CoV-2	Viral Replication	-	11.67 µg/mL	0% CPE (at 4 µg/mL)	[93]
cationic crosslinked CDs	Omicron variant	Vaccine Nanoplatfrom	-	-	Complete Protection	[94]

First and foremost, the utilization of CDs as direct antiviral agents has emerged as one of the most extensively investigated therapeutic strategies. CDs aimed to inhibit viral entry

rely on their tunable surface chemistry, are characterized by a variety of functional groups based on the choice of the precursor materials, and have facile post-synthesis functionalization in order to promote interactions with the viral spike protein or host ACE2 receptor. On the other hand, CDs that target viral replication interfere with intracellular viral processes, mainly through inhibition of N-CTD dimerization. Although both approaches fall under the same broad class of direct antiviral strategies of CDs, they target different stages of the viral life cycle. Moreover, from a mechanistic perspective, the nature of interactions taking place between CDs and viral components is also different, based on the physicochemical and structural properties of the target. Notably several studies have explored the combination of both mechanisms to achieve a synergistic antiviral effect. Unlike antiviral CDs, which suppress viral infection through direct inhibition of viral entry or replication, vaccine nanoplateforms utilize CDs as antigen carriers to enhance immune activation. To the best of our knowledge, the study of Lei et al. [94] is the only reported *in vivo* therapeutic approach, suggesting complete protection against the Omicron variant of COVID-19 in animal models. This may be attributed to the early stage of development of CD-based antiviral therapeutics, the need for high-containment animal facilities for SARS-CoV-2 research, and the lack of standardized CD formulations for preclinical evaluation. Consequently, most studies remain at the proof-of-concept stage and focus primarily on *in vitro* validation.

The above CD-based therapeutic strategies offer several advantages. Starting from the protagonist CDs, these nanomaterials belong to the class of advanced carbon-based nanostructures with low or negligible toxicity, and facile large-scale production from abundant carbon-based precursors, making them promising candidates for sustainable and cost-effective antiviral interventions. The application of CDs as direct antiviral agents targeting viral entry results in rapid and efficient inhibition of viral infection through interference with the early stage of the viral life cycle, while the risk of intracellular toxicity is lower since action is mostly extracellular. Simultaneously, the diversity of available synthesis and characterization techniques of CDs allows precise control of functional groups and size, thereby optimizing antiviral performance and blocking a wide range of viruses. Even after infection has occurred, CDs could act at post-entry stage inhibiting viral replication through the alteration of enzymes that are needed for viral genome replication, while a broader antiviral spectrum may be achieved when highly conserved replication-associated targets are involved. In the reported literature, porphyrin and curcumin-derived CDs have been investigated as representative examples of viral replication inhibition, not only due to their intrinsic antiviral properties, but also due to their enhanced hydrophilicity. As vaccine nanoplateforms, CDs contribute to enhanced antigen stability and strong humoral and cellular immune responses. Overall, these strategies highlight the multifunctional role of CDs in antiviral applications.

Despite the promising features, several limitations still remain that hinder the clinical application of CDs as both antiviral agents and vaccine nanoplateforms. Among these, the most significant is the dependence of the physicochemical properties of CDs on both synthesis method and precursor materials, resulting not only in considerable variability between the reported studies, but also posing a major challenge to the reproducibility and standardization of CD-based systems. The substantial variability in CD synthesis methods, structural properties, and reported antiviral metrics, along with the lack of standardized protocols have made the comparison across various studies complicated, thereby limiting the identification of the most effective CDs formulation. Furthermore, although CDs are proposed to interfere with viral entry, intracellular replication processes, or even multimodal antiviral mechanisms involving ROS generation and membrane disruption, the precise molecular interactions underlying these antiviral effects have not been investigated and remain incompletely understood. Although the CD-based vaccine nanoplateforms currently

exhibit the highest level of preclinical validation among the reported CD-based therapeutic strategies, the absence of human studies evaluating the long-term safety, immunogenicity, and efficacy restricts their clinical application.

Consequently, the reported findings indicate that CD-based therapeutic strategies could be a promising solution against COVID-19; nevertheless, further efforts toward standardization, mechanistic validation, biocompatibility and toxicity assessments, and clinical applications are required so that these nanoplateforms could be considered viable therapeutic options.

7. Challenges and Future Perspectives

Despite the development of CD-based platforms for the detection and treatment of COVID-19, several challenges should be addressed before their efficient clinical translation. These challenges refer to the synthesis of nanomaterials, diagnostic aspects, and therapeutic aspects, and they highlight the need for further investigation.

First and foremost, scaling up the synthesis of CDs remains a major challenge from both scientific and technological standpoints, as it requires precise control over size, surface chemistry, and biocompatibility to ensure consistent performance for biomedical applications. At the same time, critical parameters related to CDs production, including: i. synthesis routes (top-down or bottom-up approaches), ii. synthetic conditions (e.g., reaction temperature and time, pH control), iii. precursor compounds (e.g., carbon source, doping agents) and iv. surface passivation, should be carefully optimized to improve reproducibility, performance, and industrial viability. Future efforts should focus on improving batch-to-batch reproducibility and developing standardized synthetic protocols capable of producing CDs with consistent physicochemical characteristics and biological performance. These advances are essential to facilitate industrial production and clinical translation [95]. In particular, there is a variety of top-down and bottom-up synthesis routes, as well as organic compound precursors employed to produce CDs, resulting in products with different surface chemistry, particle size, and physicochemical properties. In addition, heteroatom doping of CDs leads to substantial differences in optical responses, and along with the aforementioned factors, the antiviral efficacy of the CDs is consequently varied. This variability impedes the direct comparison among the studies and poses further concerns regarding the approval of CD-based antiviral platforms for diagnostic devices or biomedical formulations. Despite the remarkable progress achieved in laboratory-scale synthesis, translating CD production into a robust, reproducible, and economically viable industrial process remains a major scientific and technological challenge. Moreover, since the available literature remains difficult to compare systematically, the establishment of a reliable structure–activity relationship is limited, posing a significant barrier to the industrialization and commercialization of CD-based technologies.

Several research studies underline the enhanced antiviral performance of CDs, which could be attributed to disruption of virus membrane, binding to a spike protein or to nucleocapsid inhibition. However, the exact interaction mechanisms between CDs and viral components remain vague and plausible [87,92]. The majority of the studies propose a mode of action based on *in silico* analysis or *in vitro* models. Regarding the diagnostic applications of CDs, the origin of PL tuning upon viral recognition is not always elucidated, rendering the sensor optimization and reproducibility difficult [93]. Moreover, the antiviral activity of CDs is highlighted based on purified viral proteins or pseudoviruses, which may not fully replicate complex clinical samples. The demonstration of activity at a cellular level is important, yet it should not be directly translated to *in vivo* effectiveness due to physiological barriers, metabolism, and dose limitations. The low cytotoxicity of CDs has encouraged further investigation into their *in vivo* biocompatibility, immunogenicity, and

biodistribution. However, these aspects remain largely unexplored despite being critical parameters for the clinical application of CD-based platforms, particularly with respect to dose optimization, systemic exposure, and long-term safety [95,96].

Furthermore, thorough examination of CD metabolism patterns is vital to avoid potential accumulation in organs. Numerous *in vivo* studies have addressed notable variations in tumor accumulation and retention times in animal models. Surface chemistry is considered a key parameter of the metabolic profile of CDs, since their carbon core possesses high structural stability; therefore, CDs are resistant to metabolic degradation and primarily eliminated from the body in an intact form. As a result, it is crucial to either modify or eliminate specific surface functional groups of CDs with strong affinity toward biological macromolecules to regulate clearance. The size of CDs also plays a major role in the following metabolism pathway. Owing to their ultrasmall dimensions, CDs can be successfully eliminated from systemic circulation, predominantly through renal or hepatic routes. However, in case of larger particles or those bearing surface functionalities with strong affinity toward organs, such as the heart, lungs, or brain, they tend to exhibit prolonged retention in these tissues. In particular, renal filtration barriers can manage CDs with diameters up to 8 nm, whereas the liver processes CDs with larger diameters that remain in circulation. Metabolism of CDs is also influenced by particle charge, since fenestrated endothelium of the kidney is negatively charged, excretion of positively charged molecules is fostered. In case of negatively charged particles, the liver has a major role in metabolism. Surface chemistry of CDs represents a determining factor for their metabolism because hydrophobic functional groups of CDs should be oxidized, reduced, or hydrolyzed with the aid of hepatic microsomal enzymes to facilitate their clearance via renal or hepatic routes. The metabolic behavior of CDs is also affected by the route of administration. Typically, CDs are delivered via subcutaneous, intravenous, or intratumoral injections. Among them, intravenous injection enables rapid systemic distribution and minimizes premature interactions with biomolecules such as proteins, hormones, vitamins, or plasma components that could alter the surface chemistry and physicochemical properties of CDs [8,97]. From a regulatory perspective, it is considered essential to establish standardized characterization protocols, validated manufacturing procedures, and comprehensive preclinical evaluation of toxicity, biodistribution, pharmacokinetics, and immunogenicity. Regulatory approval will further depend on compliance with Good Manufacturing Practice (GMP), Good Laboratory Practice (GLP), and internationally accepted guidelines for nanomedicines issued by regulatory agencies such as the Food and Drug Administration (FDA) and European Medicines Agency (EMA). Despite the encouraging preclinical findings reported up to date, it should be mentioned that no CD-based antiviral diagnostic or therapeutic platform has proceeded to routine clinical practice or obtained regulatory approval.

Looking forward, the future of CD-based nanoplatforms in COVID-19 management is directed towards the rational design of multifunctional systems that integrate diagnostic and therapeutic capabilities [98]. The choice of precursor compounds, either commercially or biomass-derived, can modulate surface chemistry and further boost their antiviral performance or sensing ability. In addition, surface functionalities can determine the dual role of CDs in ROS generation or scavenging, acting as antiviral agents or mitigating inflammation, respectively. Furthermore, machine-learning (ML) tools are expected to play a transformative role in the design and scalable synthesis of CDs. Specifically, ML algorithms could be vital tools in establishing a relationship between structure and properties by correlating precursor composition, reaction conditions, particle size, heteroatom doping, and surface functionalities with antiviral efficacy and biosensing performance. These predictive models aim to facilitate experimental procedure, which is currently based on trial-and-error approaches that are considered time-consuming and resource-intensive concepts. Also, the

integration of ML tools with high-throughput experimentation and automated synthesis platforms could lead to the development of novel CDs with increased reproducibility and scalable manufacturing [95]. For diagnostic applications, future efforts should be directed towards the improvement of sensitivity and selectivity of CD-based biosensors in complex biological environments. Integration with microfluidic platforms, lateral flow assays, and smartphone-based readout systems could further enhance clinical applicability [99]. Upon a therapeutic aspect, extensive pharmacokinetic and immunological evaluations could provide a safety and efficacy profile for CDs implemented as therapeutic agents or versatile carriers in vaccine delivery, offering long-lasting protection from SARS-CoV-2 variants. Overall, continued interdisciplinary research combining materials science, virology, medicine, and clinical studies is expected to unlock the full potential of CDs as vital components for the development of next-generation theranostic tools in the combat of COVID-19 and future viral outbreaks.

8. Conclusions

In summary, this review article presented the application of CDs as both diagnostic and therapeutic tools towards COVID-19, addressing the role of synthesis, functionalization, optical properties, and antiviral mechanisms in the design of advanced multifunctional materials for emerging viral pandemics. CDs appeared as highly versatile nanomaterials with superb photoluminescence, increased biocompatibility, and tunable surface chemistry, providing avenues in the theranostics of COVID-19. In diagnostic applications, CDs stand out as fluorescent biosensors based on turn-on and turn-off mechanisms, as well as FRET phenomenon, showing their ability to detect with sensitivity and low LOD viral components, even in real biological samples. Additionally, CDs act as diagnostic tools for COVID-19 by modifying commonly used electrodes and amplifying their electrochemical signals. In each case, the use of immunorecognition elements, including SARS-CoV-2 spike protein and anti-N protein antibodies, offer new perspectives in the development of CD-based immunosensors with remarkable selectivity and sensitivity. Except for diagnostic approaches, CDs demonstrated their ability to act as therapeutic agents in antiviral nanoplatforms by inhibiting viral entry, replication, and nucleocapsid dimerization, as well as modulating oxidative stress and supporting vaccine-delivery strategies, highlighting their multifunctional role. Both the antiviral and sensing performance of CDs is highly influenced by synthesis conditions, surface functionalization, particle size, and redox behavior. Biodistribution, long-term safety studies, and standardized, reproducible preparation protocols for CDs with controlled properties are essential steps towards their clinical translation. Overall, the intrinsic fluorescence, increased biocompatibility, and multifunctional surface chemistry position CDs as promising nanomaterials with the potential to integrate diagnosis and therapy and to strengthen preparedness for future viral pandemics.

Author Contributions: Conceptualization, A.K., A.Z. and K.V.K.; Writing—Original Draft Preparation, A.K., A.Z., A.T. and N.B.; Writing—Review and Editing, Z.S. and K.V.K.; Supervision, Z.S. and K.V.K. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the European Union's Horizon Europe Research and Innovation Program, under Grant Agreement No. 101058422 (SUPREME). Alexandra Karagianni acknowledges funding from the Special Account for Research Funding (E.L.K.E.) of the National Technical University of Athens (N.T.U.A.), Greece.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflicts of interest.

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