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Commentary

Carbon nanostructure: Cutting-edge platforms for advanced genetic transport

Sara Yazdani[1], Mehrdad Mozaffarian[1,*], Gholamreza Pazuki[1], Naghmeh Hadidi[2]

[1]Department of Chemical Engineering, Amirkabir University of Technology, Tehran P.O. Box 15875-4413, Iran

[2]Department of Clinical Research and EM Microscope, Pasteur Institute of Iran (PII), Tehran, 1316943551, Iran

*Author for correspondence:
Email: mozaffarian@aut.ac.ir

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Abstract

Gene therapy represents a groundbreaking approach to treating genetic disorders, cancers, and infectious diseases by directly modifying or correcting the genetic material within a patient's cells. Unlike conventional therapies, which primarily alleviate symptoms, gene therapy addresses the root cause of diseases at the molecular level, offering the potential for long-term or even permanent cures. However, the success of this method depends on the development of safe, efficient, and targeted delivery systems that can overcome multiple biological barriers. Among non-viral vectors, carbon nanostructures such as carbon nanotubes (CNTs), carbon quantum dots (CQDs), and nanodiamonds (NDs) have emerged as promising candidates due to their high surface area, biocompatibility, and ability to be easily functionalized. Despite these advantages, obstacles such as low gene delivery efficiency and potential toxicity still hinder their widespread clinical use. This article reviews recent progress in the development of carbon nanostructures for gene delivery and discusses ongoing challenges. Continued advancements in this field have the potential to reshape gene therapy, bringing it closer to clinical reality.

Keywords: Carbon nanomaterials, Advanced gene delivery systems, Non-viral vectors carriers

Abbreviations: CNTs: Carbon Nanotubes; CQDs: Carbon Quantum Dots; NDs: Nanodiamonds; SWCNTs: Single-Walled Carbon Nanotubes; MWCNTs: Multi-Walled Carbon Nanotubes; CVD: Chemical Vapor Deposition; pDNA: Plasmid Deoxyribonucleic Acid; mRNA: Messenger Ribonucleic Acid; siRNA: Small Interference RNA; miRNA: Micro RNA; GQDs: Graphene Quantum Dots; anti-PSMA: Anti-Prostate-Specific Membrane Antigen; CNDs: Carbon Nanodots; PDs: Polymer Dots; CDs: Carbon Dots; UNCD: Ultra-Nanocrystal Diamond; NCD: Nanocrystalline-Diamond; (HPHT): High Pressure High Temperature; PEI: Polyethyleneimine

Introduction

Gene therapy represents a revolutionary approach to treating a wide range of genetic disorders, cancers, and infectious diseases by directly modifying or correcting defective genes within a patient's cells. Unlike conventional treatments that focus on managing symptoms, gene therapy targets the underlying molecular causes of diseases, offering the potential for long-lasting or permanent cures. Over recent years, significant advancements in gene therapy have been made, with several clinical trials and therapies showing promising results [1,2]. For instance, in cystic fibrosis, gene therapy is being developed to correct mutations in the CFTR gene, which is responsible for the disease's characteristic thick mucus production in the lungs [3]. Similarly, gene therapy has shown potential in treating hemophilia, a genetic blood-clotting disorder, by replacing defective genes to restore the production of clotting factors [4]. Additionally, in sickle cell anemia, gene therapy is being explored to modify stem cells and promote the generation of normal, healthy blood cells [5]. Certain cancers, including leukemia, are also being targeted by gene therapies that modify immune cells (such as CAR-T cell therapy) to specifically attack cancer cells [6]. Despite these advances, the need for innovative approaches to improve the performance of carbon nanostructures in gene delivery systems is more pressing now

than ever. Current research focuses on refining these nanomaterials through surface modifications, enhancing their physicochemical properties, and developing novel functionalization techniques that will improve their stability, targeting efficiency, and safety profiles [7,8]. By tailoring the design of these nanostructures, researchers are aiming to overcome major delivery obstacles, including immune recognition, cellular uptake limitations, endosomal escape, and the precise release of genetic material within targeted cells [9]. Among the various vectors explored for gene delivery, CNTs, CQDs and NDs have garnered significant attention due to their high surface area, biocompatibility, and ease of functionalization [10]. CNTs have been studied for their potential in delivering plasmid DNA (pDNA) and siRNA, offering promising solutions for gene therapies targeting disorders such as hemophilia and certain cancers. Similarly, NDs have been utilized in microRNA and siRNA delivery, showing success in reprogramming pluripotent stem cells into cardiac cells and effectively reducing tumor cell proliferation in cancer therapies [10]. These emerging studies highlight the potential of carbon nanomaterials to overcome the existing barriers in gene therapy, particularly in delivering genetic material safely and efficiently into target cells [10]. As we stand on the cusp of a new era in genetic medicine, understanding and refining the clinical applications of these nanostructures is crucial for advancing gene therapy and unlocking its full potential.

Carbon Nanotubes (CNTs)

CNTs are cylindrical nanostructures composed of rolled graphene sheets made from carbon atoms arranged in a hexagonal lattice. Discovered by Iijima [11] in 1991, CNTs have become prominent in nanotechnology due to their unique structure and remarkable properties, such as exceptional tensile strength, high electrical and thermal conductivity, chemical stability, and extraordinary flexibility [10,12,13]. Their lengths vary significantly, ranging from nanometers to centimeters, bridging the gap between molecular and macroscopic scales. CNTs are primarily classified into Single-Walled Carbon Nanotubes (SWCNTs) and Multi-Walled Carbon Nanotubes (MWCNTs), with SWCNTs consisting of a single graphene layer and MWCNTs composed of multiple concentric layers [14]. This structural variation influences their properties, resulting in different synthesis method requirements and potential applications. The diameter of SWCNTs typically ranges from 0.4 to 3 nm, while those of MWCNTs are thicker, with diameters spanning from 1 to 200 nm. CNTs exhibit extraordinary mechanical strength, high electrical and thermal conductivity, and excellent flexibility, making them valuable in numerous applications, including drug and gene delivery systems. Their large surface area allows for the attachment and transport of various therapeutic molecules, while their chemical stability and resilience to withstand extreme temperatures further enhances their versatility [15]. Despite these advantages, challenges such as toxicity and low solubility remain, necessitating application of functionalizing techniques that improve biocompatibility, and enable their safe application in biomedical applications [13,16]. CNTs can be synthesized by various methods, with each one requiring key components such as an active catalyst, a carbon source, and sufficient energy [17]. The most prominent synthetic techniques include arc-discharge [18], laser ablation [19], and chemical vapor deposition (CVD) [20]. The arc-discharge method utilizes a pair of graphite electrodes in a low-pressure helium atmosphere, generating CNTs on the cathode as the anode is consumed. This method typically produces MWCNTs, though SWCNTs can also be synthesized with this technique by introducing metallic catalysts [21]. The laser ablation method, which involves vaporizing solid graphite with a high-intensity laser, offers a high yield of high-quality SWCNTs, but requires substantial energy input, making it less economical [22]. Lastly, the CVD method employs a carbon-containing gas such as methane or acetylene, which decomposes on a metal catalyst at high temperatures, allowing for a controlled growth of CNTs with precise alignment and diameter [23]. Despite their successful synthesis, the as-produced CNTs often exhibit undesirable properties, including low solubility and high toxicity, which can hinder their biomedical applications. Therefore, functionalization of CNTs through covalent or non-covalent methods is essential to enhance their dispersion, biocompatibility, and overall applicability in various fields [24,25]. The arc-discharge method is a straightforward approach that produces high-quality CNTs, but it has limited scalability and generates a considerable amount of by-products, raising environmental concerns [18]. The laser ablation technique, while yielding high-purity CNTs with precise control over their properties, is expensive and energy-intensive, which restricts its scalability [19]. On the other hand, CVD is the most scalable and cost-effective method for large-scale CNT production, but it typically results in lower purity CNTs and often requires post-synthesis treatment, contributing to its environmental impact [20]. CNTs are promising tools in gene therapy due to their unique properties, such as high surface area, biocompatibility, and the ability to be easily functionalized. Pure CNTs are inherently hydrophobic, high toxicity and struggle to disperse in aqueous solutions, but surface modification—through either covalent or non-covalent functionalization—enhances their solubility and biocompatibility [26,27]. This modification introduces functional groups such as carboxyl or amine, making CNTs effective carriers for various genetic materials, including plasmid DNA, siRNA, miRNA, aptamers, and antisense oligonucleotides. Various studies have demonstrated their application in gene delivery; for example, CNTs functionalized with ammonium have been shown to deliver plasmid DNA with minimal cytotoxicity, while CNTs modified with hexamethylenediamine and poly(diallyldimethylammonium) chloride provided efficient transportation of siRNA via electrostatic interactions [28]. Additionally, MWCNTs functionalized with polyethylene glycol and linked to anti-prostate-specific membrane antigen (anti-PSMA) aptamers were successfully utilized to target prostate cancer cells, demonstrating enhanced delivery efficiency in both *in vitro* and *in vivo* models. These examples highlight the broad potential of functionalized CNTs in enhancing gene therapy through targeted and efficient nucleic acid delivery [29,30]. Chen and colleagues [30] modified SWCNTs by incorporating amylose derivatives containing poly(L-lysine) dendrons (ADP@SWNT). Their research focused on evaluating parameters such as the stability of aqueous dispersions, cytotoxicity, gene transfection efficiency, and the photothermal properties of the complex both in vitro and in vivo. In another study, Yazdani *et al.* [31] non-covalently functionalized SWCNTs using DSPE-PEG-COOH and demonstrated their effectiveness as a delivery system for amphotericin B, while also exploring their potential for gene material delivery. Pantarotto *et al.* [28] modified CNTs with ammonium groups to facilitate plasmid DNA (pDNA) delivery, noting that the functionalized CNTs exhibited low cytotoxicity and were able to easily penetrate cells. Similarly, Krajcik *et al.* [32] chemically functionalized SWCNTs with hexamethylenediamine (HMDA) and poly(diallyldimethylammonium) chloride (PDDA) to create a nanocarrier capable of binding siRNA via electrostatic

interactions, concluding that PDDA-SWNT was an effective system for intracellular siRNA delivery.

Carbon Quantum Dots (CQDs)

CQDs are a class of zero-dimensional carbon nanoparticles with dimensions typically under 10 nm, and distinguished by their robust fluorescence properties. Discovered in 2004 by Xu *et al.* [33] during purification of SWCNTs, CQDs have since gained prominence due to their superior water solubility and ease of preparation compared to other carbon nanomaterials like carbon nanotubes and nanodiamonds. CQDs include subcategories such as graphene quantum dots (GQDs), carbon nanodots (CNDs), and polymer dots (PDs), with each varying in internal structure but generally featuring mono-disperse spherical shapes with carbon cores and surface functional groups such as oxygen[34]. These nanoparticles exhibit exceptional chemical stability, low toxicity, and good electrical conductivity. CQDs are known for their strong photoluminescence and enhanced solubility, primarily due to carboxyl groups on their surfaces, which also contribute to their significant optical properties. These traits make CQDs highly suitable for applications in biomedicine, including bio-imaging and bio-sensing, as well as in drug and gene delivery systems. Various studies have demonstrated their high biocompatibility and efficacy in cancer therapy, with CQDs showing promising results in reducing cancer cell viability and improving therapeutic outcomes. Functionalization of CQDs with biocompatible molecules further enhances their utility as nanocarriers, offering the potential for advanced drug delivery and cancer treatment strategies [35,36].

The synthesis of CQDs involves various methods that can be broadly categorized into top-down and bottom-up approaches [37]. Top-down methods, such as laser ablation, ultrasonic synthesis, and arc-discharge typically involve the breakup of larger carbon materials such as carbon nanotubes, soot, or graphite into nanoscale elements CQDs [38]. For instance, laser ablation uses high-energy laser beams to produce CQDs with high water solubility and fluorescence, although size control remains challenging [39]. Ultrasonic methods leverage high-frequency sound waves to create nanoscale CQDs from bulk carbon materials, offering simplicity and cost effectiveness [40]. Electrochemical carbonization is another top-down technique that employs electrical currents to generate CQDs from bulk carbon sources under ambient conditions. This method produces CQDs with varying colors and sizes, depending on the electrolytes and conditions used [41]. Bottom-up methods, including hydrothermal synthesis, microwave irradiation, and thermal decomposition, start from small organic molecules and build up CQDs through chemical reactions [42]. Hydrothermal treatment uses high pressure and temperature to convert precursors such as citric acid or glucose into CQDs, yielding high quantum yields and uniform sizes [43]. Microwave irradiation is a rapid method that generates CQDs from organic solutions such as those containing citric acid, offering good optical properties and low cytotoxicity [44]. Thermal decomposition involves heating organic materials to produce CQDs, which is a straightforward approach that can be scaled up for large production. Each method has its advantages and limitations in terms of cost, scalability, and control over CQD properties, making the choice of synthesis method dependent on the desired application and CQD characteristics [45,46]. CQDs have emerged as promising tools in gene therapy due to their unique properties and versatile applications. Their large surface area-to-volume ratio, photoluminescence, low toxicity, eco-friendliness, good water solubility, and biocompatibility make them ideal for various biomedical applications [47]. Functionalization has enhanced their capabilities, such as the one conducted by Lo and colleagues' [48], involving modification of GQDs with polyethylenimine, which improved their biocompatibility and enabled efficient delivery of green fluorescent protein to colon cancer cells by enhancing cell membrane penetration. Similarly, N-doped GQDs have been used to form electrostatic complexes with mRNA or plasmids, facilitating effective gene transfection. Şimşek *et al.* [49], synthesized carbon dots (CDs) from Nerium Oleander extract that could penetrate cell nuclei and interact with genes, potentially causing DNA damage. Hasanzadeh *et al.* [50]created zinc/nitrogen-doped CDs via a microwave method, which effectively delivered both large plasmids and mRNA into HEK-293 cells, showcasing high transfection efficiency and notable photoluminescence. Zhang *et al.* [51] developed CDs from citric acid and panteaethylenehexamine, rich in amine groups, which demonstrated low toxicity and high transfection efficiency. Liu *et al.* [52], further enhanced gene delivery by functionalizing GQDs with PEI for miRNA delivery to hepatocarcinoma cells. *In vivo* applications are also promising, such as the ones by Huang *et al.* [53], synthesizing photo-stable CQDs for bio-imaging and potential phototherapy, and Wang *et al.* [54], developing polymer-coated nitrogen-doped carbon nanodots with adjustable fluorescence, showing high accumulation in glioma for fluorescent imaging. However, considerations of toxicity and stability are crucial, as Liu *et al.* [55] found out that increased light intensity and temperature could lead to greater degradation of CQDs and potential toxicity. Overall, CQDs offer significant potential for targeted gene therapy and bio-imaging, with ongoing research expected to refine their applications and improve their performances.

Nanodiamonds (NDs)

Diamond nanoparticles, or NDs, were first synthesized using a detonation technique in 1960 [56]. These particles are composed of a diamond core surrounded by a layer of amorphous carbon with carbon atoms arranged in both sp^3 and sp^2 hybridization states. Typically, NDs diameters are less than 20 nm [57]. Their unique properties, including exceptional hardness, high Young's modulus, remarkable optical characteristics, fluorescence, resistance to harsh environments, and high thermal conductivity, make them highly attractive for a wide range of applications. Additionally, NDs exhibit intrinsic biocompatibility, low toxicity, stable fluorescence, and a large surface area, which enhances their suitability for biomedical applications such as drug and gene delivery, bio-imaging, and bio-sensing [58]. They can effectively carry a variety of therapeutic materials and exhibit low toxicity across different cell lines and animal models. Bio-distribution studies have revealed that NDs do not accumulate significantly *in vivo* over time. Despite their hydrophobic nature and tendency to agglomerate in aqueous solutions, techniques such as sonication, mechanical grinding, and surface modification with biocompatible polymers or biological molecules can improve their dispersion and functionality. Surface functionalization with various groups, notably carboxylic acids, enhances their dispersibility, biocompatibility, and capacity to conjugate with other materials or biological molecules, enabling targeted applications with customized properties [59-61].

NDs can be produced using various synthesis methods, with each influencing their properties differently. The CVD technique,

developed in the 1970s, is known for its ability to produce large and highly pure diamonds [62]. This method avoids additional purification and allows for precise control over luminescent centers, although it is costly and has a slow deposition rate, with potential toxicity concerns. CVD can generate Ultra-nano-crystal diamond (UNCD) and nano-crystalline-diamond (NCD) films with specific microstructures [63,64]. The High Pressure - High Temperature (HPHT) method, introduced in the 1960s [65], transforms graphite into diamonds under extreme pressure and temperature conditions, producing NDs with significant toughness, thermal stability, and wear-resistance. HPHT allows for accurate size control, and can incorporate doping elements such as silicon or nitrogen [66,67]. The Detonation Technique, which uses explosive forces to convert graphite into diamonds, is cost-effective and simple, producing NDs with high hardness and biocompatibility. However, this method often produces impurities like graphite and amorphous carbon, necessitating additional purification steps [68,69]. Thus, each synthesis technique imparts distinct characteristics to NDs, impacting their suitability for various applications. NDs are emerging as highly promising nano-scale carriers for gene therapy due to their adjustable surface chemistry, high biocompatibility, and effective dispersibility. They can be modified with cationic polymers like polyethyleneimine (PEI) and polyamidoamine to enhance their ability to load and deliver negatively charged nucleic acids, although safety concerns regarding these polymers in clinical use need to be addressed [70]. NDs can significantly boost gene delivery efficiency and biocompatibility, and sometimes improving delivery yields of up to 70 times greater than conventional methods [71,72]. To enhance stability and delivery, NDs can be combined with niosomes—which are lipid-based vectors that protect and release genetic materials safely. Studies have demonstrated that ND-PEI and ND-siRNA complexes can efficiently deliver genetic material *in vivo* and have shown their effectiveness in reducing tumor cell proliferation and promoting cellular reprogramming [73-75]. Additionally, ND-based systems have been used to deliver small molecule inhibitors such as UNC0646 for cancer treatment, improving their solubility and therapeutic efficacy [76]. Overall, NDs hold significant potential for advancing gene therapy, although further research is needed to fully understand issues such as their interactions with cells, cytotoxicity, and biodistribution. Xu and colleagues [75] developed complexes of nanodiamonds (NDs) and siRNA through electrostatic interactions. Their findings demonstrated that NDs were more efficient than liposomal formulations in delivering siRNA to tumor cells, leading to a significant reduction in tumor cell proliferation [75]. Liu *et al.* [77] designed a nanodiamond-based system for microRNA delivery, which effectively promoted the reprogramming of pluripotent stem cells into a myocardiogenic lineage. Gu and coworkers [76] utilized NDs as a carrier for inhibitors at an in vivo level, forming ND-UNC0646 complexes through physical adsorption. They observed that this formulation enhanced the dispersibility of the UNC0646 inhibitor, making it suitable for intravenous administration.

Comparative Summary of Carbon Nanostructures in Gene Therapy

Carbon nanostructures, including CNTs, CQDs, and NDs, each exhibit distinct properties that make them suitable candidates for gene therapy applications. CNTs are characterized by their exceptional electrical conductivity, high surface area, and mechanical strength, which facilitate efficient loading and delivery of genetic material [78]. Their ability to penetrate cell membranes, coupled with various functionalization methods (e.g., covalent and non-covalent approaches), enhances their biocompatibility and reduces toxicity [79]. In contrast, CQDs are notable for their excellent photoluminescence and biocompatibility, making them ideal for both imaging and therapeutic purposes. They possess a relatively smaller size compared to CNTs, which can improve cellular uptake and reduce potential cytotoxic effects [80]. Finally, NDs are distinguished by their excellent biocompatibility and stability in biological environments. Their nanoscale size and ability to be functionalized through surface modifications allow for effective gene delivery and targeting [81]. When comparing these three nanostructures, CNTs excel in mechanical properties and gene loading capacity, CQDs shine in imaging capabilities and low toxicity, while NDs offer significant advantages in biocompatibility and stability, underscoring the importance of selecting the appropriate nanomaterial based on specific therapeutic requirements [10].

Conclusion

In summary, researchers around the globe are excited about the potential of non-viral nanostructures to revolutionize gene therapy. Future advancements may lead to more affordable treatments, replacing costly options and introducing new genetic therapies for a range of diseases. Like other biologics, gene therapies are expected to see significant progress, particularly with the optimization of carbon delivery systems for targeted applications. To enhance the effectiveness of gene therapies, future research should focus on improving the biocompatibility and efficacy of carbon nanostructures in clinical settings, including investigating their interactions with various cell types and the immune system. These nanostructure-based delivery systems are emerging as promising solutions, addressing existing challenges and fulfilling diverse biological and medical needs. By ensuring active delivery of genetic materials into cells, rather than relying on passive mechanisms, the field of nano-gene delivery is harnessing innovative nanostructures to develop effective vectors for various applications. The unique properties of carbon-based vectors offer potential solutions for challenges in delivering genetic materials both *in vitro* and *in vivo*. Overcoming the current limitations of these systems could significantly transform gene therapy practices, making treatments more efficient, targeted, and accessible. Although interest in this area is increasing, research is still in its early stages, necessitating further exploration of nanostructures and their biological interactions to fully realize their potential in gene therapy.

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Conflict of Interests

The authors declare no conflict of interest.

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