Carbon-Based Nanomaterials: Multifunctional Materials for Biomedical Engineering

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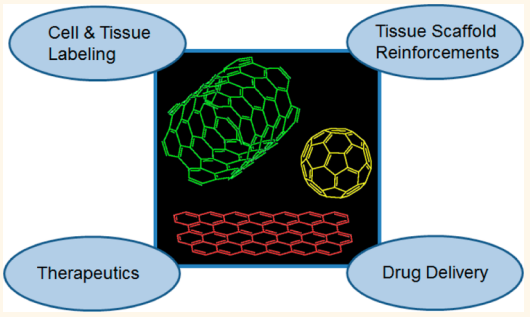
ABSTRACT Functional carbon-based nanomaterials (CBNs) have become important due to their unique combinations of chemical and physical properties (*i.e.*, thermal and electrical conductivity, high mechanical strength, and optical properties), and extensive research efforts are being made to utilize these materials for various industrial applications, such as high-strength materials and electronics. These advantageous properties of CBNs are also actively investigated in several areas of biomedical engineering. This Perspective highlights different types of carbon-based nanomaterials currently used in biomedical applications.



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Graphite is one of the oldest and most widely used natural materials. More traditionally known as the main ingredient of pencil lead, from which the name "graphite" originated, it is now more widely used in several large-scale industrial applications, such as carbon raising in steelmaking, battery electrodes, and industrial-grade lubricants.[1] Due to its high demand, the consumption of synthetic graphite has significantly increased in recent years. Extensive scientific investigation into graphite has revealed that its unique combination of physical properties stems from its macromolecular structure, which consists of stacked layers of hexagonal arrays of sp^2 carbon.[1]

With the deeper appreciation and development of nanofabrication techniques and nanomaterials that have progressed within the last two decades, graphite is now being actively used as a starting material to engineer various types of carbon-based nanomaterials (CBNs), including single or multiwalled nanotubes, fullerenes, nanodiamonds, and graphene (Figure 1).[2] These CBNs possess excellent mechanical strength, electrical and thermal conductivity, and optical properties; much of the research efforts have been focused on utilizing these advantageous properties for various applications, such as high-strength composite materials and electronics.[1,2]

The field of biomedical engineering has also embraced the growing popularity and influence of CBNs in recent years because many of its applications rely heavily on the performance of biomaterials. Carbon-based nanomaterials have been widely regarded as highly attractive biomaterials due to their multifunctional nature. In addition, incorporating CBNs into existing biomaterials could further augment their functions. Therefore, CBNs have found their way into many areas of biomedical research, including drug delivery systems, tissue scaffold reinforcements, and cellular sensors.[3]

Carbon Nanotubes. Since their discovery, carbon nanotubes (CNTs) have become the most widely used CBNs.[4,5] Carbon nanotubes are commonly synthesized by arc discharge or chemical vapor deposition of graphite. They have a cylindrical carbon structure and possess a wide range of electrical and optical properties stemming not only from their extended sp^2 carbon but

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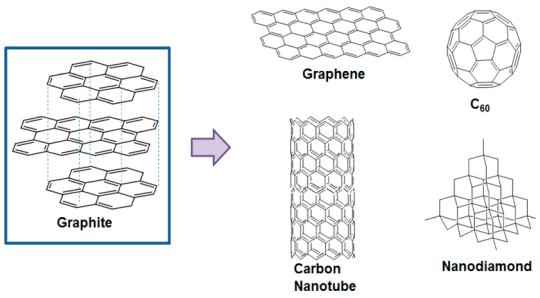

Figure 1. Various types of carbon-based nanomaterials.

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also from their tunable physical properties (*e.g.*, diameter, length, single-walled *vs* multiwalled, surface functionalization, and chirality).[5] Due to their diverse array of useful properties, CNTs have been explored for use in many industrial applications.[6] For example, CNTs are well-known for their superb mechanical strength: their measured rigidity and flexibility are greater than that of some commercially available high-strength materials (*e.g.*, high tensile steel, carbon fibers, and Kevlar). Thus, they have been utilized as reinforcing elements for composite materials such as plastics and metal alloys, which have already led to several commercialized products.[7] However, the possibility of CNT-incorporated composites as super high-strength load-bearing materials has not been met with satisfactory results, mostly due to their poor interaction with the surrounding matrices, which leads to inefficient load transfer from the matrices to the CNTs.[7]

Many recent research efforts have been geared toward incorporating CNTs into various materials to utilize their multifunctional nature (*i.e.*, electrical and thermal conductivity and optical properties) rather than focusing purely on composite mechanical strength. For example, the excellent electrical properties of CNTs coupled with their nanoscale dimensions are of great interest in electronics for the construction of nanoscale electronic circuitry.[8,9] In addition, CNTs are known to have low threshold electric fields for field emission, as compared with other common field emitters.[10,11] Thus, CNTs are actively explored in high-efficiency electron emission devices such as electron microscopes, flat display panels, and gas-discharge tubes. Carbon nanotubes also display strong luminescence from field emission, which could be used in lighting elements.[9]

Carbon Nanotubes in Biomedical Engineering. There is considerable interest in using CNTs for various biomedical applications. The physical properties of CNTs, such as mechanical strength, electrical conductivity, and optical properties, could be of great value for creating advanced biomaterials.[3] Carbon nanotubes can also be chemically modified to present specific moieties (*e.g.*, functional groups, molecules, and polymers) to impart properties suited for biological applications, such as increased solubility and biocompatibility, enhanced material compatibility, and cellular responsiveness.[12] Their applications include cell and tissue labeling agents, injectable drug delivery systems, and biomaterial reinforcements.

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Cell and Tissue Labeling and Imaging. The possibility of using CNTs as labeling and imaging agents has been discussed since their discovery due to their unique optical properties. Carbon nanotubes have optical transitions in the near-infrared (NIR) region, which has been shown to be useful in biological tissue because NIR has greater penetration depth and lower excitation scattering.[3] In addition, fluorescence in the NIR region displays much lower autofluorescence than do the ultraviolet or visible ranges. These properties make CNTs potent imaging agents with higher resolution and greater tissue depth for NIR fluorescence microscopy and optical coherence tomography. For example, Cherukuri *et al.* successfully monitored CNTs in phagocytic cells and those intravenously administered into mice using NIR fluorescence.[13]

Raman spectroscopy has been used extensively to characterize the structural features of CNTs, as they are highly sensitive to Raman scattering because of their extensive symmetric carbon bonds.[14] The characteristic Raman signatures of CNTs have also been utilized as cellular probes. For example, Liu *et al.* detected CNTs in various tissues after intravenous delivery into mice using Raman spectroscopy.[15]

Drug Delivery Systems. Drug delivery has benefited greatly from the advances in nanotechnology by using a variety of nanomaterials (*i.e.*, liposomes, polymersomes,

microspheres, and polymer conjugates) as vehicles to deliver therapeutic agents.[16] Carbon nanotubes have also been investigated extensively as drug delivery systems since CNTs have been shown to interact with various biomacromolecules (*i.e.*, proteins and DNA) by physical adsorption.[17] In addition, several chemical modification schemes have been developed to conjugate therapeutic molecules or targeting moieties covalently to CNTs.[12]

In one interesting study, Zheng *et al.* provided important insight into the interactions between CNTs and DNA molecules.[18] Carbon nanotubes were shown to be effectively dispersed in aqueous media in the presence of single-stranded DNA (ssDNA). Spectroscopic and microscopic analyses provided evidence of strong interactions between DNA molecules and CNTs, resulting in individual dispersion. Molecular dynamics modeling showed that the base of ssDNA interacted with the surface of CNT *via* $\pi-\pi$ stacking, resulting in helical wrapping of ssDNA chains around the CNT (Figure 2). This research highlights the potential use of CNTs for gene delivery, as well as DNA-specific separation techniques for molecular electronics.

Reinforcing Tissue Engineering Scaffolds. The use of CNTs as composite reinforcements for tissue engineering scaffolds to date has primarily been focused on enhancing their mechanical properties.[19] Commonly used scaffold materials, such as hydrogels and fibrous scaffolds, are inherently soft in order to mimic the stiffness of natural tissues and thus often lack structural strength and support. Incorporating CNTs into these materials has been shown to enhance their mechanical properties. For example, Shin *et al.* demonstrated that incorporating CNTs into photo-cross-linkable gelatin hydrogels resulted in significant increases in their tensile strengths (Figure 3).[20] Sen *et al.* also demonstrated improvement in the tensile strengths of CNT-reinforced polystyrene and polyurethane fibrous membranes.[21]

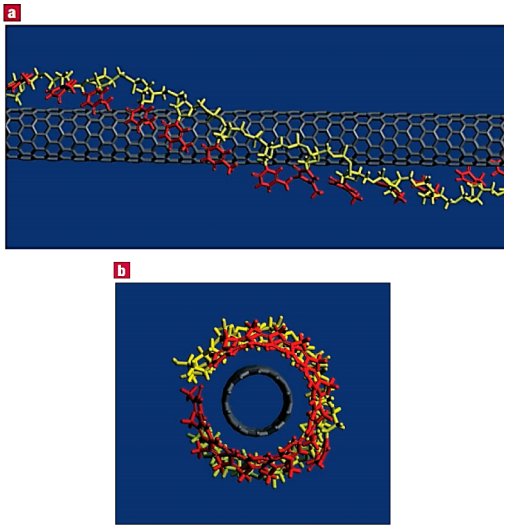

Figure 2. Binding model of a carbon nanotube (CNT) with a poly(T) DNA sequence. The DNA wraps around the CNT in a right-handed helical structure. The bases (red) orient to stack with the surface of the nanotube and extend away from the sugar–phosphate backbone (yellow). The DNA wraps to provide a tube within which the CNT can reside, hence converting it into a water-soluble object. Reprinted with permission from ref 18. Copyright 2003 Nature Publishing Group.

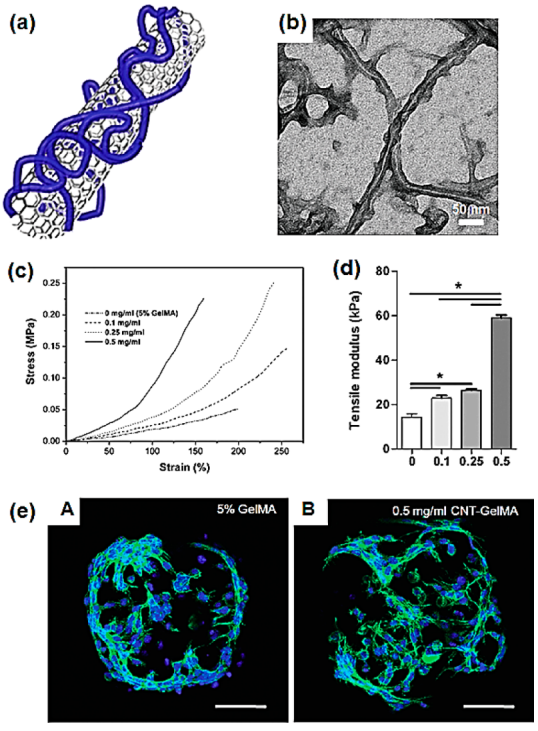

Figure 3. (a) Schematic description of gelatin methacrylate (GelMA) coated onto a carbon nanotube (CNT). (b) High-resolution TEM image of CNT-GelMA. (c) Stress–strain curves of CNT-GelMA hydrogels with various concentrations of CNTs. (d) Tensile moduli of CNT-GelMA hydrogels. (e) 3T3 fibroblasts cultured on GelMA hydrogel (A) and CNT-GelMA hydrogel (B) (scale bar: 100 μm). Reprinted from ref 20. Copyright 2011 American Chemical Society.

More recently, researchers have turned their attention to utilizing the multifunctional nature of CNTs in engineering tissue scaffolds. Most notably, CNTs have been incorporated to fabricate electrically conductive scaffolds. Most of the biomaterials used for tissue engineering

applications are electrically insulating, as they are made from nonconductive polymers.[22−24] However, certain applications, such as neural and cardiac tissues, would benefit greatly from conductive scaffolds that can effectively propagate electrical signals across the tissue constructs for proper electrophysiological functions. For example, Kam *et al.* applied electrical stimulation to neural stem cells (NSCs) grown on CNT−laminin composite films and demonstrated improved action potentials of NSCs and differentiation into functional neural networks.[23] Shin *et al.* cultured cardiomyocytes on CNT-reinforced gelatin hydrogels where the cells showed enhanced electrophysiological activities and ultimately developed functional cardiac tissue.[25] These works highlight the ability of CNTs to impart electrical conductivity successfully to otherwise nonconductive biomaterials.

Cytotoxicity of CNTs. Despite many successful applications in biomedical engineering, there is a growing concern for safety with CNTs. Some recent *in vitro* studies have reported increased cytotoxicity of CNTs due to their cellular uptake, agglomeration, and induced oxidative stress.[26−28] These conflicting results regarding the biocompatibility of CNTs largely stem from the variability of CNTs (*i.e.*, size, surface properties, and functionalization) and testing protocols (*i.e.*, *in vitro vs in vivo*, types of cells, tissues, and animals tested). In addition, increased cytotoxicity has often been attributed to incomplete removal of metal catalysts used to prepare CNTs.[27] Most *in vivo* studies using CNTs have shown insignificant toxicity and reported renal clearance from the body, although small portions of CNTs have been shown to accumulate in certain organs, such as lungs, liver, and spleen and may cause inflammation.[26−28] However, the cytotoxicity seems to be more highly variable and more pronounced at the cellular level, based on several *in vitro* cell culture studies.[29,30]

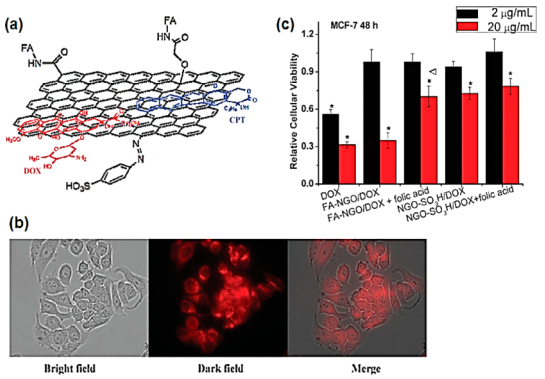

Figure 4. (a) Graphene oxide (GO) was funtionalized with folic acid (FA) as a targeting molecule (FA-NGO), then loaded with anticancer drug, doxorubicin (DOX) or camptothecin (CPT). (b) FA-NGO was localized into MCF-7 (human breast cancer) cells, identified with fluorescent labeling with rhodamine. (c) Greater anticancer activity of DOX was observed when FA-NGO was used as a drug carrier. Reprinted with permission from ref 39. Copyright 2010 Wiley-VCH Verlag GmbH & Co.

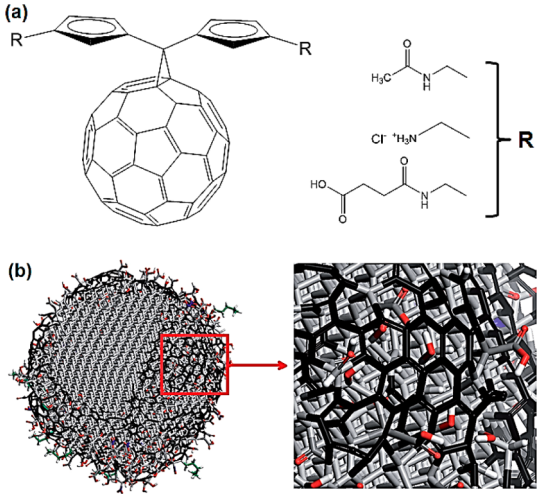

Figure 5. (a) Class of C_{60} derivatives that display anti-HIV activity.[44] (b) Molecular structure of a 5 nm diameter nanodiamond (ND). The ND is mostly made up of sp^3 carbon, but the outer layer is functionalized with sp^2 carbon and other functional groups. Reprinted with permission from ref 45. Copyright 2012 Nature Publishing Group.

With the continued growth of CNTs in various biomedical fields, more systematic biological evaluations of CNTs having various chemical and physical properties are warranted in order to determine their precise pharmacokinetics, cytotoxicity, and optimal dosages. Nevertheless, many studies have shown that toxicity can effectively be minimized by functionalizing the CNTs with biocompatible polymers or surfactants to prevent aggregation.[31,32]

Graphene. Graphene is the latest nanomaterial to burst onto the scene. The ground-breaking work by Geim and Novoselov provided a simple method for extracting graphene from graphite *via* exfoliation and explored its unique electrical properties.[33,34] Graphene and CNTs possess similar electrical, optical, and thermal properties, but the two-dimensional atomic sheet structure of graphene enables more diverse electronic characteristics; the existence of quantum Hall effect and massless Dirac fermions help explain the low-energy charge excitation at room temperature and the optical transparency in infrared and visible range of the spectrum.[34] In addition, graphene is structurally robust yet highly flexible, which makes it attractive for engineering thin, flexible materials.[35,36]

Graphene in Biomedical Engineering. Research into utilizing graphene for

Several biomedical applications including injectable cellular labeling agents, drug delivery systems, and scaffold reinforcements have been explored using graphene oxide.

biomedical applications has been limited to date, as graphene research itself is still in its infancy. Graphene oxide (GO), produced by oxidation of graphite under acidic conditions, is more commonly used, as it offers several advantages over using pure graphene.[37] First, GO is dispersible in aqueous media, which is essential for biological applications. Second, GO presents hydrophilic functional groups that enable chemical functionalization. Third, GO has broader ranges of physical properties than pure graphene due to its structural heterogeneity. Several biomedical applications including injectable cellular labeling agents, drug delivery systems, and scaffold reinforcements have been explored using GO, as has similarly been done with CNTs.[38] For example, Zhang *et al.* functionalized GO with folic acid (FA) as a cancer-targeting molecule and loaded doxorubicin and camptothecin, known cancer drugs, onto the large surface area of GO via $\pi-\pi$ stacking (Figure 4).[39] The drug-loaded FA-GO showed improved cancer-targeting capability and anticancer activity as compared with drugs delivered alone or drugs carried with unmodified GO. Sun *et al.* also demonstrated that poly(ethylene glycol)-conjugated GO with targeting molecules could be used as a cellular sensor by utilizing the intrinsic photoluminescence property of GO at the NIR region.[40] In another study, Zhang *et al.* incorporated GO into poly(vinyl alcohol) hydrogels to improve their mechanical strength.[41]

Other Carbon-Based Nanomaterials. Buckminsterfullerene (C_{60}), also commonly known as the buckyball, is a spherical closed-cage structure (truncated icosahedron) made of 60 sp^2 carbon. Its discovery in 1985 and subsequent investigation led to the uncovering of electronic properties, stemming from its highly symmetrical structure, and potential applications, culminating in a Nobel Prize in 1996.[42] It can be argued that the scientific pursuit of CBNs and their potential applications began with the discovery of C_{60}. The popularity of C_{60} has somewhat diminished in recent years with the rise of more scalable and practical CBNs such as CNTs and graphene. However, its uniform size and shape as well as availability for chemical modification led many scientists to develop C_{60} derivatives for therapeutic purposes.[43] Perhaps the most fascinating and highly promising aspect of C_{60} is its anti-human immunodeficiency virus (HIV) activity. Schinazi *et al.* first discovered a group of water-soluble C_{60} derivatives capable of inhibiting HIV protease activity by binding to its active site, due to their unique molecular structure and hydrophobicity (Figure 5a).[44] Various C_{60} derivatives have since been developed that display anti-HIV activity by targeting other important HIV enzymes, such as reverse transcriptase.[43] These results demonstrate that C_{60} derivatives may become a potent group of AIDS therapeutics in the future.

Nanodiamond (ND) has also generated interest in the field of biomedical engineering in recent years (Figure 5b).[45] Nanodiamonds are synthesized by high-energy treatment of graphite, most commonly *via* detonation, and are smaller than 10 nm. They have similar physical properties as bulk diamond, such as fluorescence and photoluminescence, as well as biocompatibility. Unlike other CBNs, NDs are made up mostly of tetrahedral clusters of sp^3 carbon. The surface of nanodiamonds, however, is functionalized with various functional groups or sp^2 carbon for colloidal stability, which enables chemical modification for targeted drug and gene delivery and tissue labeling. For example, Lien *et al.* recently used fluorescent and magnetic NDs for cell labeling.[46] Zhang *et al.* also demonstrated that polyethyleneimine (PEI)-conjugated NDs were highly effective as gene carriers, without the cytotoxicity associated with PEI alone.[47]

CONCLUSIONS AND FUTURE OUTLOOK

Extensive research efforts over the last two decades have elevated the status of CBNs as one of the most widely used classes of nanomaterials. Owing to their unique combinations of mechanical, optical, and electrical properties, CBNs have been explored in various industrial applications. Several areas of biomedical engineering have also benefited greatly from CBNs in recent years because incorporating CBNs is effective not only as injectable nanoscale devices but also as components to enhance the function of existing biomaterials significantly. Despite safety concerns over CBNs, many studies have reported the successful use of CBNs in biological applications. In addition, several chemical modification strategies have been developed to circumvent toxicity issues and to increase the biocompatibility and functionality of CBNs. Nevertheless, it should be noted that more systematic toxicology studies are needed to determine the toxicity and pharmacokinetics of CBNs.

This paper has introduced several successful applications of CBNs in drug delivery, tissue imaging, and scaffold reinforcement. With the popularity of CBNs as highly versatile and useful nanomaterials, we expect to see continued use of CBNs in many facets of biomedical engineering. In particular, there is great promise in applying the biocompatible and multifunctional nature of CBNs to the areas that interconnect mechatronics and biology, such as microelectromechanical systems

("MEMS") for biological sensors and actuators.[48] Furthermore, more recent studies suggest that CBNs may also be used to regulate cellular behavior.[49,50] Although research efforts have largely been focused on utilizing CNTs, other types of CBNs—especially graphene, which has gained wide recognition in recent years—are expected to be investigated extensively in the near future.

Conflict of Interest: The authors declare no competing financial interest.

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