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15 Nanodiamond: Designing the Bio-Platform

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Introduction

All major forms of carbon at the nanoscale – fullerenes, nanotubes, and nanodiamond (ND), the last in the forms of both particulate and films – appear to be valuable materials for biomedical applications (Freitas, 1999; 2003). Importantly, carbon nanostructures span the same length scale as bio-compounds (Fig. 15.1), ranging from subnanometer-size nucleotides, to tens and hundreds of nanometer-sized organelles and viruses, and up to micron-sized cell sizes.

In the mid 1990s it was discovered that fullerene compounds have biological activity, and their potential as therapeutic agents for the treatment of several diseases was demonstrated. As a result, a private biopharmaceutical company, C Sixty Inc., has been established with a primary focus on the discovery and development of novel fullerene-based therapeutics. At 7.2 Å in diameter, C$_{60}$ is similar in size to steroid hormones and peptide alpha-helices, and, thus, fullerene compounds are ideal molecules to serve as ligands for enzymes and receptors (Wilson, 2000). Within the last few years, a number of useful fullerene-based therapeutic applications have been developed, including as antiviral agents and anticancer drugs (www.csixty.com) and biosensors for diagnostic applications (Anonymous, 2001); a protective agent against iron-induced oxidative stress (Lin et al., 1999); and an in vitro antibacterial agent (Da Ros et al., 1996).

The exploration of buckytubes in biomedical applications is also underway. Multiwall carbon nanotubes have been used for immobilization of proteins, enzymes, and oligonucleotides (Lin et al., 2004). Significant progress has been made within the last few years in an effort to overcome some of the fundamental and technical barriers toward bioapplications of carbon nanotubes, especially on issues concerning solubility in water, biocompatibility, modifications of carbon nanotubes with various biological and biologically active compounds, and both design and fabrication of biosensor prototypes (Lin et al., 2004).
Nanodiamond particles and their derivatives, diamondoids and their derivatives, and ultrananocrystalline diamond (UNCD) films all have high potential in biotechnological and biomedical applications. UNCD films have been suggested to be the ideal platforms for future biochips and biosensors because of their superior mechanical, thermal, and chemical properties as compared to those of glass, silicon, and gold surfaces (Yang et al., 2002). UNCD particles had been probed in the separation of proteins (Bondar and Puzr, 2004), fabrication of integrated biochips and sensors (Puzyr et al., 2004a), and even in anticancer applications (Dolmatov, 2001).

In general, multifunctional hierarchical structures consisting of nanoparticle derivatives of organic and inorganic molecular entities began to play increasingly important roles in a variety of applications in nanobiotechnology (Niemeyer, 2001), genomics (Wengel, 2004), drug discovery (Ozkan, 2004), and nanomedicine (Freitas, 2003). For example, zero-dimensional nanostructures reported for medical applications include gold and magnetic nanoparticles, semiconductor quantum dots, a wide variety of polymer-based nanoparticles, nanoshells consisting of metals and dielectrics, and many others. Nanoparticles act as potential carries for...
several classes of drugs such as anticancer agents, antihypertensive agents, immunomodulators, and hormones; and macromolecules such as nucleic acids, proteins, peptides, and antibodies. An absence of narrow fractions of nanodiamond particulate on the market hindered their biomedical applications. Recently, however, production of narrow fractions of ND (5 nm sized particle suspensions; several fractions within the 40–100 nm size range as well as fractions above 100 nm) has been achieved in research laboratories (Chapter 3 of this book and private communications). In combination with the advances in the production of high-purity particles and the fact that UNCD particles of detonation origin are relatively inexpensive (compared to fullerenes, pure carbon nanotube (CNT), and gold particles, for example), a fast growth of bioapplications of ND particulate is expected.

This chapter will be organized as follows: in the next section different approaches to the surface functionalization of ND particles, that is, the key in successful biomedical applications, will be summarized, followed by a discussion of modification of diamond surfaces with nucleic acids and proteins. After that both current and potential applications of diamond films and particles in the areas of biosensing and medicine will be addressed. The concluding section will summarize results reported on the biocompatibility of ND, the paramount property in the biomedical applications of artificial nanostructures.

### 15.1 Functionalization of ND with Heteroatoms and Chemical Groups

Diamond possesses a number of distinct properties that make it an attractive biotechnological material. Although natural diamond is highly hydrophobic, hydrophilic surface groups, important for bioapplications, can be generated on a diamond surface by heating to high temperature in an oxygen atmosphere and using ion bombardment and other rather aggressive treatments. ND particles synthesized by detonation have numerous oxygen-containing chemical groups on the surface and, thus, are intrinsically hydrophilic. The detonation ND particles are 4–5 nm round-shaped monocrystals that form tightly and loosely bond aggregates of about 50 nm and 100–200 nm in diameter, correspondingly. Detonation ND possesses a very large specific surface area (300–400 m$^2$ g$^{-1}$) (Dolmatov, 2001), which is an important factor for bioapplications. Detonation ND is likely to have a significant number of unpaired electrons that make it an efficient free radical scavenger and opens up the
opportunity for its use in medicine (Kulakova et al., 2000; Nachalnaya et al., 2000).

In general, the size, purity, and surface chemistry of detonation ND varies considerably from one manufacturer to another. The composition of the explosive mixture, coolant media, chamber size, and consequent purification of detonation soot are all important factors in the manufacture of UNCD particles with the desirable physical and chemical properties of their surface (Donnet et al., 1997). The methods of extraction and purification of ND from the detonation product may include ozone treatment, oxidation by different reagents with/without catalysts, treatment with acids, modification of the ND surface in gaseous and liquid media (Kulakova, 2004), selective inhibition of ND oxidation (Chiganov, 2004), and many others. Disaggregating of detonation ND can be achieved, for example, by its graphitization in a N₂ atmosphere at 1000 °C followed by oxidation in air at 450 °C in order to remove the surface graphite layer. In this way, the average size of ND aggregates can be reduced to 50 nm or less with a very high yield (Xu and Xue, 2004).

Dispersion efficiency and stability of colloidal solutions of ND in water is important for ND applications in nanobiotechnology and medicine, and is determined by the electrostatic, hydration, and hydrophobic (the tendency of lyophilic ND to form aggregates in an aqueous solution) interactions among the particles (Xu et al., 2005a). Within the last few years a number of new methods have been introduced to control ND solubility in water by biological and chemical modifications of the ND surface. To obtain stable suspensions of well-dispersed ND particles in water the mechanochemical procedure has been developed, which employs a set of different mechanical treatments (high-power sonification or vibration milling techniques) along with the application of surfactant agents (Xu et al., 2005b; 2005c). The mechanochemical modification with anionic surface modifiers increased the zeta potential and lowered the amount of hydroxyl groups and the size of individual particles (Zhu et al., 2004). The mechanochemical processes were also successfully applied for the preparation of stable highly dispersed ND suspensions in non-polar solvents (Xu et al., 2004): an oil suspension of ND particles with an average size of 55 nm was prepared and stored for 6 months without any visible signs of sedimentation.

There has been steady progress in the development of chemical methods of ND surface modification as well. A summary of methods of altering the ND surface chemistry with the final purpose of biofunctionalization is illustrated in Fig. 15.2. Various organic molecules can be attached to polycrystalline diamond films when under irradiation with UV
light. Prior to exposure to UV light the hydrogen-terminated diamond surface must be coated with a thin film of the solution containing organic molecules in order to be immobilized. By attaching molecules with specific protecting groups and removing protecting groups after the attachment, it is possible to obtain diamond surfaces functionalized with carboxyl or primary amine groups that may facilitate further steps in chemical modification of diamond surfaces such as DNA and protein immobilization (Strother et al., 2002).

To increase ND solubility in water and prepare the ND surface for biomolecule attachment, oxidation of the ND surface is frequently required (Fig. 15.2). Under mild conditions the diamond surface can be directly functionalized with carboxylic acids by initiating radical reactions (Tsubota et al., 2004). However, the number of carboxylic acid residues introduced on the diamond surface may be low in some cases. Indeed, oxidation of ND does not proceed easily under the mild conditions. In an aqueous slurry, ND of 4 nm size may react with ozone, though a very long time is needed in order to functionalize the surface of ND with the ketonic groups (Cataldo and Koscheev, 2003). In air ND may suddenly explode.
above a temperature of 450°C. The functionalization of NDs at elevated temperature affects both their size and surface chemistry. The weight of ND particles was reported to decline by 11.5% as a result of heating at 900°C in an inert atmosphere (Cataldo and Koscheev, 2003). Spherical diamond powder with a particle size ranging from 150 to 600 nm can be oxidized in an oxygen atmosphere at 450–610°C (Lee et al., 2004). The oxidized diamond powders were further successfully functionalized by reacting with 4-(trifluoromethyl)benzylamine containing the reactive amino group (Lee et al., 2005). Under certain conditions the high rate of diamond oxidation may result in an explosive process. Ida et al. (2003) have investigated the reactivity of hydrogenated diamond surfaces with peroxide radical initiators such as benzoyl peroxide, lauroyl peroxide, dicumyl peroxide, and di-t-butyl peroxide. With benzoyl peroxide, they detected IR peaks that were then assigned to the aromatic C—H and C—O stretching vibrations. The C—O stretching vibration was observed after the diamond was exposed to lauroyl peroxide. The areas under the peak of spectra determined with Fourier transform infrared spectroscopy (FTIR) increased with reaction time and amount of reagent. Dicumyl peroxide and di-t-butyl peroxide did not react with diamond surfaces. While the yield of reaction of diamond surface with radical species generated from benzoyl peroxide depends on the organic solvent used, the same functional groups were synthesized in toluene, tetrahydrofuran, N,N-dimethylformamide, cyclohexane, and hexane (Tsubota et al., 2002a). The hydroxyl groups located on the surface of diamond powders, which was treated either with sulfuric acid alone or with a mixture of sulfuric and nitric acids, can be reacted with metoxy groups of silane-coupling reagents (3-aminopropyltrimethoxysilane, 3-mercaptopropyltrimethoxysilane, or n-octyltrimethoxysilane) with the formation of stable modified diamonds (Tsubota et al., 2002b). The silane-modified NDs can be used for surface synthesis of DNA and protein immobilization.

The substantial increase in the number of surface C—H groups was achieved by the gas treatment of ND powder with H₂, plasma-ionized hydrogen, N₂, methane and air, and in vacuum at different temperatures (Jiang et al., 1996). Surface decomposition, decarbonylation, and decarboxylation were likely to be the main reactions. The H-terminated ND surfaces can be further reacted photochemically (λ = 254 nm) with long-chain ω-unsaturated amines to produce a homogeneous layer of amine groups for consequent DNA attachment (Yang et al., 2002).

ND purification and functionalization can also be carried out using gas and vapor reactive media (Fig. 15.2). The chlorinated diamond was pre-
pared by Sotowa et al. (2004) by irradiating hydrogenated diamond with UV light in the presence of elemental chlorine. The formation of chlorinated diamond was confirmed by diffuse reflectance FTIR, which revealed a strong peak corresponding to the C—Cl stretching. The researchers then treated the chlorinated diamond surface further with ammonia and found that diamond amination is a temperature-dependent process and results in the formation of NH$_4^+$; C≡N and NH$_2$; and imines at room temperature, 100 °C, and 200 °C, respectively. High-temperature treatment of detonation ND with hydrogen, CCl$_4$, or NH$_3$ has been studied recently by Spitsyn et al. (2005). ND, which was annealed in hydrogen flow at 850 °C for 5 h, possessed about 1000 cal g$^{-1}$ lower combustion heat than non-modified ND powder. Moreover, the initial dangling bonds density of $\sim$1.16 × 10$^{20}$ spin/cm$^3$ was reduced 1.5 times after the treatment with hydrogen. After treatment in a CCl$_4$/Ar mixture at 450 °C for 0.5–3 h the hydrophilicity of ND was changed significantly: the atmospheric water vapor readsoorbance was at least 20 times lower than in the ND samples treated in pure Ar. After treatment in a NH$_3$ flow at 600 °C for 70 min the number of oxygen-containing groups had decreased and atmospheric water vapor readsoorbance was four times lower than in the initial ND samples. A number of characterization methods such as chemical analysis, Raman, FTIR, electron spin resonance (ESR), and chromatomass spectrometry confirmed the possibility of extensive modification and controlled functionalization of the ND using gas treatment (in terms of hydrophilic/hydrophobic or acidic/basic ND termination) (Spitsyn et al., 2005). This prevents agglomeration of hydrophilic and hydrophobic detonation ND in polar and non-polar solvents, correspondingly.

Fluorination is another efficient method for ND chemical modification that enables a variety of applications in engineering and biological sciences (Fig. 15.2). Treatment of detonation UNCD powder (1–2 μm sized particles composed of 3.5–6.5 nm diamond nanocrystals, >97% purity) with a F$_2$/H$_2$ mixture at 150–470 °C resulted in the formation of fluorinated NDs with 8.6 at.% fluorine (Liu et al., 2004). The fluorinated ND material was then used as a precursor for preparation of alkyl-, amino-, and amino acid-functionalized NDs that showed an increased solubility in polar solvents and reduced particle agglomeration (Liu et al., 2004). Application of fluorinated ND has been found in cost-effective synthesis of diamond coatings covalently bonded to glass surfaces (Liu et al., 2005). Before that the production of diamond thin films could only be achieved by chemical vapor deposition that requires heating to 1000 °C. Liu et al. (2005) applied a silane coupling agent, 3-aminopropyltriethoxysilane, to attach fluoro-ND to the glass slide surface, which was preliminary func-
tionalized with terminal amino groups. Using atomic force microscopy (AFM), SEM, and X-ray photoelectron spectroscopy (XPS) analysis it was established that surface-bonded fluoro-ND particles were closely packed and had an average size of 10–40 nm. The fluoro-ND, which is covalently attached to the surface of the glass slide, can be further modified by chemical substitution of residual fluorine.

15.2 Modification with Nucleic Acids

Nanoparticles are valuable platforms in controlled drug delivery and can be administered via most routes to carry various therapeutics, anti-cancer, antiviral, antibacterial, and antihypertensive agents, immunomodulators, hormones, antibodies, proteins, peptides, and nucleic acids to isolated cells, tissues, and organs (Bala et al., 2004; Ozkan, 2004). Successful design of highly efficient drug delivery systems may solve many problems faced by present-day medical sciences.

In gene therapy, genes are delivered to the cell nucleus allowing cells to produce therapeutic proteins. Gene delivery is achieved using either viral or non-viral vectors. Viral vectors are genetically engineered adenoviruses, retroviruses, and other viruses that are very efficient when used for gene transfer in vivo. Methods of non-viral gene delivery, including nanoparticle carriers, represent a small fraction of all methods used for transfection in vivo. However, they have gradually become as popular as the viral vector-based technology. In the development of non-viral technologies, the main goal is to have a transfection method that is efficient, reproducible, non-toxic, and allows the prolonged gene expression to occur. Efficiency of targeted delivery of nanoparticles loaded with biologically active molecules is affected by many factors including particle size, surface charge, and chemistry, and mechanism of target recognition. Over the years, a number of natural and synthetic materials have been used to prepare nanoparticles, and their stability, biocompatibility, and biodegradability were investigated (Bala et al., 2004). In some cases nanocarriers protect naked DNA from nucleases while allowing DNA plasmids to pass the cell membrane and get into the nucleus (Kneuer et al., 2000; Roy et al., 2005). To optically monitor intracellular trafficking and gene transfection events Roy et al. (2005) first prepared fluorescently labeled organically modified silica nanoparticles for use in non-viral gene delivery and biophotonics applications and then showed that these nanoparticles can serve as a delivery platform with superior efficacy in targeted drug therapy and as the real-time monitoring of drug action. The highly monodispersed stable water
suspensions of the organically modified silica nanoparticles, which were labeled with the fluorescent dyes and functionalized by amino groups, were prepared using micelle chemistry. The nanoparticles efficiently bound DNA due to positively charged amino groups and protected it from digestion by DNase I. Imaging by fluorescence confocal microscopy confirmed that \textit{in vitro} cells efficiently took up the nanoparticles in the cytoplasm, and the nanoparticles delivered DNA to the nucleus. Very recently Bejjani et al. (2005) reported the breakthrough discovery that polylactic nanoparticles enable \textit{in vivo} gene transfer and expression with a high efficiency. Solid nanoparticles can be used to deliver drugs and biologically active molecules to any body organ including the brain, because they can cross the blood–brain barrier (Lockman et al., 2002; Koziara et al., 2003). The nanoparticles that are biodegradable in a controlled way are likely to become the carriers of choice for \textit{in vivo} non-viral gene delivery. Surface-modified and appropriately labeled ND particles may also serve as an efficient platform for \textit{in vivo} transfection. However, because of the very low, if any, biodegradability of ND, ND nanoparticles, when applied \textit{in vivo}, must be of a size small enough to allow their excretion by the kidney or applied cutaneously.

Immobilization of DNA on diamond surfaces via covalent bonding has been explored intensively. For example, Ushizawa et al. (2002) first modified diamond powder with particle sizes of 1–2µm (Fig. 15.3) by oxidation in a heated mixture of sulfuric acid and nitric acid and then converted it in chlorocarbonyl–diamond by reacting with thionyl chloride at 50°C for 1 day. Chlorocarbonyl–diamond was then reacted with thymidine in the presence of 4-dimethylaminopyridine. The DNA was attached to the 3'-end of diamond-attached thymidine by 5'-end phosphatization. The formation of ester bonds was confirmed by diffuse reflectance FTIR spectroscopic analysis. Preparation of DNA-modified diamond films for use in hybridization has received increased attention. The chemical stability of diamond surfaces is substantially greater than that of gold or silicon surfaces (Lu et al., 2004), and the DNA molecules attached to the diamond surfaces are easily accessible to enzymes. Nanocrystalline diamond thin films covalently modified with DNA oligonucleotides following the photochemical modification of H-terminated surfaces with amine groups provide a very stable and highly selective platform for the surface hybridization reaction (Yang et al., 2002). After linking DNA to the amine groups, hybridization reactions with fluorescently tagged complementary and non-complementary oligonucleotides did not reveal any non-specific adsorption, with extremely good selectivity between matched and mismatched sequences (Yang et al., 2002). In a similar manner, hydrogen-
terminated diamond substrates were photochemically converted into amine-terminated surfaces following by linking to thiol-terminated DNA oligonucleotides reported by Knickerbocker et al. (2003). The DNA hybridization on DNA-modified polycrystalline diamond is highly specific and when compared to the hybridization on DNA-modified surfaces of crystalline silicon shows that the diamond surface exhibits superior chemical stability (Knickerbocker et al., 2003). DNA-modified diamond surfaces are particularly suitable for invasive cleavage reactions, in which introduction of target DNA to solution results in the specific cleavage of surface-bound probe oligonucleotides, permitting SNP (single nucleotide polymorphisms) detection. The sensitivity of the analysis can be improved 100 times by replacing the DNA-modified gold surface with a more stable DNA-modified diamond surface (Lu et al., 2004). CVD diamond has also found some applications for DNA immobilization. Using thymidine as a

![Diagram of DNA immobilization on diamond surface](image-url)

**Figure 15.3** Immobilization of DNA on diamond surface. (a) thymidine-immobilized diamond powder is obtained by diamond surface carboxylation (acid treatment), followed by formation of chlorocarbonyl surface groups reacting with thymidine in anhydrous pyridine. (b) DNA-ligated diamond through thymidine ester. (From Ushizava et al., 2002, with permission.)
linker molecule, the fragment of the human PKU gene was covalently bound to the CVD diamond film by Wenmackers et al. (2003).

Boron-doped diamond (BDD) thin films with enhanced conductivity offer a substantial advantage for use in DNA hybridization analysis. For example, Gu et al. (2004) electropolymerized a thin layer of polyaniline/poly(acrylic acid) onto the diamond surface. The carboxylic acid residues in the polymer film enhanced the electron transfer between DNA and a BDD surface and acted as the binding sites for DNA attachment. Both fluorescence microscopy and cyclic voltammograms indicated that the polymer-modified BDD did not show significant non-specific DNA adsorption, while providing a stable transduction platform for DNA detection by hybridization.

The absence of efficient technology for in vivo delivery of oligonucleotides limits many therapeutic applications. For successful application in vivo, in most cases in vitro delivery platforms should be extensively modified in order to provide targeted drug delivery. The adamantane-based materials have found application in the preparation of carriers for in vivo nucleic acid delivery because they can be modified by using cyclodextrin/adamantane host/guest interactions to provide the particles suitable for systemic application (Pun and Davis, 2002). Transferrin-modified nanoparticles containing DNAzymes (DNA enzymes that are RNA-cleaving phosphodiester-linked DNA-based enzymes, which cleave their target mRNA in a gene-specific fashion) for targeting tumors were prepared by using conjugates of adamantane with poly(ethylene glycol) and administered to tumor-bearing nude mice by intraperitoneal bolus and infusion, intravenous bolus, and subcutaneous injection. DNAzymes packaged in polyplex formulations were concentrated and retained in tumor tissue, whereas unformulated DNAzyme was eliminated from the body within 24 hours after administration (Pun et al., 2004). In wound healing therapy, the localized delivery of growth factors is achieved by gene transfer to the wound site. Synthetic biocompatible materials prepared with a linear, beta-cyclodextrin-containing polymer and an adamantane-based crosslinking polymer are very suitable for in vivo gene delivery to fibroblasts via the inclusion of adenoviral vectors in the synthetic construct (Bellocq et al., 2004). Gene-deleted adenoviral vectors were originally developed as delivery vehicles for use in gene therapy trials and are currently being developed as HIV vaccines and in other medical applications.

From a different perspective, in biological materials science and in rapidly emerging angstrom-scale chemical engineering the nucleic acids are thought to find many applications in the fabrication of self-assembling,
multi-dimensional materials (Wengel, 2004). ND and diamondoid structures can be valuable candidates for crosslinking of oligonucleotides in carbon-based systems.

### 15.3 Interaction with Proteins

Both selective adsorption of proteins and their immobilization onto surfaces of ND particles (Fig. 15.4) may be advantageous in nanobiotechnological applications and medicine.

Fibrinogen is widely accepted as an indicator in a biocompatibility test and material-caused inflammation. The adsorption of human fibrinogen on the surface of chemical-vapor-deposited diamond has been studied by Tang et al. (1995) and was the first report on the diamond interaction with

![Diagram](image)

**Figure 15.4** Role of hydroxyl-, carboxyl-, and amino groups in possible immobilization of proteins on nanodiamond. (a) Covalent linkage of enzyme ND surface by coupling of carboxy functionalities; (b) attachment of enzyme via 2,4,6-trichlorotriazine to ND surfaces; (c) siloxane assemblies on ND surfaces for the immobilization of enzymes, where Z is a complementary functional group for X; (d) covalent linkage of enzyme to ND surfaces by coupling of amino functionalities. (Adapted from Choi, 2004.)
a protein and its biocompatibility. The CVD diamond was found to be biologically compatible to the same extent as titanium and stainless steel.

In 1995 Kossovsky et al. demonstrated that surface-modified ND particles with a size ranging from 5 to 300 nm provided both conformational stabilization and a high degree of surface exposure to protein antigens and used them to generate antibodies. Recently Huang and Chang (2004) developed the universal procedure for protein immobilization onto the surface of 5 nm ND particles. It starts with ND particles with strong acids followed by modifying their surface with poly-L-lysine. Covalent attachment of proteins is then carried out by activating the amine-terminated ND to react with the heterobifunctional linker SSMCC followed by mixing with the protein. The researchers successfully immobilized both Alexa Fluor 488 dye and yeast cytochrome c using the free SH group for linkage. More information on the application of protein-modified ND in biosensing and biotechnology can be found in Section 15.5.

Hollow nanoparticles are thought to be the most suitable carriers of proteins and peptides susceptible to degradation. Many nanoparticles can be made hollow by heating at high temperature, treating with strong acids, alkali, and organic solvents, or using gentle chemical treatment to encapsulate susceptible organic molecules such as proteins, peptides, and enzymes or others inside the cavity of hollow nanoparticles (Sharma et al., 2005). ND particles produced by detonation of the mixture of trinitrotoluene and cyclomethylenetrinitramine have a tetragonal structure and according to Vereshchagin and Yurjev (2003) are hollow particles with an inner diameter of 18.94 Å and outer diameter of 25.47 Å. Yurjev et al. (2005) used synchrotron X-ray diffraction to characterize these spherical hollow detonation NDs that can be modified even further by grinding in a planetary mill.

The surface-modified NDs and diamondoids are expected to find vast application in the development of a new generation of protein delivery platforms and antigen carriers because they can be readily modified to both carry and stabilize biologically active molecules, proteins, and enzymes. In addition, these particles are rigid, biocompatible, available in different shapes, and span the subcellular size range (Fig. 15.1).

### 15.4 Application in Biosensors and Medicine

A biosensor (Fig. 15.5) generally comprises biomolecules sensing an analyte, e.g., DNA, antibody, receptor, or enzyme, and the electrochemical, optical, calorimetric, or piezoelectric transducer that detects an attach-
ment of an analyte to the biorecognition layer (Deisingh and Thompson, 2004). The physiochemical properties that determine the analytical characteristics of biosensing devices are surface characteristics of the sensing area and its size, as well as intrinsic properties of the material. In the past few years, significant advances have been made toward the development of both biosensors and biochips for single-cell analysis, detecting of pathogens and toxins (Vo-Dinh et al., 2001), and other biological and medical applications.

Diamond films occupy a special place as an electrode material in biosensors. When made sufficiently electrically conducting by boron doping, thin-film and free-standing diamond electrodes exhibit remarkable chemical resistance to etching, a wide potential window, low background current responses, mechanical stability toward ultrasound-induced interfacial cavitation, a low stickiness in adsorption processes, and a high degree of tunability of the surface properties (reviewed by Compton et al., 2003). Tatsuma et al. (2000) have examined direct electron transfer from BDD electrodes to heme peptide and horseradish peroxidase for the application to H$_2$O$_2$ biosensors. Diamond electrodes exhibit low sensitivity to interfering agents and may become a capable H$_2$O$_2$ biosensor.

Nanocrystalline diamond films can be both a platform for biofunctionalization and serve as an electrode in biosensors based on electrochemical reactions. Different proteins can be covalently attached to the hydrogen-terminated nanocrystalline diamond films modified with amino groups and remain fully functional. Hartl et al. (2004) functionalized nanocrystalline diamond electrodes with catalase and detected a direct electron transfer between the redox centre of the enzyme and the diamond electrode. Also, the electrode was found to be sensitive to hydrogen peroxide. Hydrogenated diamond can gain surface conductivity after exposure to air and by transfer doping with C$_{60}$ resulting in a significant rise
in two-dimensional conductivity (Strobel et al., 2004). The fully hydrogen terminated diamond surfaces are not pH sensitive but can gain this sensitivity after a mild surface oxidation by ozone (Garrido et al., 2005). The difference in DNA adsorption on the H- and O-terminated diamond surfaces may be useful in the nanofabrication of biosensors (Tachiki et al., 2003). Compared to multiwalled carbon nanotube-based electrodes, BDD electrodes exhibit less selective voltammetric responses to the different biomolecules and slower electron-transfer kinetics. BDD electrodes have no intrinsic selective response to L-ascorbic acid, and surface modification by anodic polarization is required to resolve L-ascorbic acid and dopamine (Poh et al., 2004). Highly conductive BDD electrodes are especially suited for electrochemical detection of nucleic acids in aqueous solutions. Their distinctive features are high reproducibility, small background currents at high positive potentials, and robustness under extreme conditions (Prado et al., 2002). Importantly, BDD electrodes can be used in analysis involving a heating step and ultrasonic treatment. Well-defined peaks, observed with tRNA, single- and double-stranded DNA, and 2′-deoxyguanosine 5′-monophosphate were directly assignable to the electrooxidation of deoxyguanosine monophosphate (Prado et al., 2002).

Polycrystalline diamond films deposited by microwave plasma CVD were incorporated in the design of four different glucose sensors by Troupe et al. (1998). While a diamond–platinum–glucose oxidase sensor was affected by the presence of electroactive chemicals in blood, usage of BDD as a conducting electrode in place of the platinum provided a strong and repeatable response to glucose. The sensor that was fabricated with the surface-modified diamond allowed attachment of biologically active molecules and electron transfer from glucose oxidase to the electrode. This approach received further development in a study by Loh et al. (2004). However, a 3,3′-diaminobenzidine-electropolymerized carbon nanotube-based electrode outperformed the diamond one in terms of selectivity and sensitivity. In another attempt to develop a ND-based biosensor Huang et al. (2004) immobilized polyclonal antibodies against *Salmonella typhimurium* and *Staphylococcus aureus* on nanocrystalline diamond films and evaluated the efficacy of immobilization by enzyme-linked immunosorbent assay (ELISA). The immobilization of antibodies and attachment of bacteria measured by SEM were more efficient on a surface of air plasma-treated diamond than on a surface of diamond treated preliminarily with the hydrogen plasma. The plasma-oxidized surface of ND was more hydrophilic and was terminated with hydroxyl and carbonyl groups.
Modified ND particles and films have been used in heterogeneous and electrochemical oxidation catalyses and related applications, and in electrochemical analysis (Bogatyreva et al., 2004; Song et al., 2004; Yang et al., 2005). Using the new amperometric biosensors designed with monocrystalline diamond and L- and D-amino acid oxidases, Stefan et al. (2004) conducted differential pulse voltammetric assay of L-pipecolic and D-pipecolic acids in serum samples with a low limit of detection. The impedance of the diamond film can be affected by DNA hybridization at the interface that induces a field effect in the diamond space-charge layer. By identifying a range of impedances, where the impedance is dominated by the diamond space-charge layer, and measuring the interfacial impedance, it is possible to directly monitor DNA hybridization. No DNA labeling is required. Frequency-dependent interfacial electrical properties of nanocrystalline diamond films that were covalently linked to DNA oligonucleotides were changed significantly in the presence of complementary DNA oligonucleotides, with only minimal changes due to the presence of non-complementary DNA oligonucleotides (Yang et al., 2004). The electrolyte–solution–gate field-effect transistors with H-terminated polycrystalline diamond surface were shown to be sensitive to Cl\(^-\) and Br\(^-\) and can find application in cystic fibrosis tests (Song et al., 2003). The capability for biomolecular recognition was provided to the highly sensitive field-effect transistor (Bio-FET) made of a nanocrystalline diamond thin film by linking human immunoglobulin G to the diamond surface. Electrical measurements showed that the Bio-FET responded specifically to the anti-IgG antibody (Yang and Hamers, 2004).

Puzyr et al. (2004a) have recently developed a prototype ND-based biochip for use in bioluminescent analysis. The biochip incorporates aluminum oxide film-adhesive layer-deposited ND particulate-luciferase, which retained substantial enzymatic activity.

It is also worth mentioning here that diamond sensor applications include high-sensitivity detection of charged particles such as protons and pions that are possible due to diamond’s high radiation tolerance. A CVD diamond strip detector that can be used for tracking and detection of charged particles has been recently introduced on the market. Fast charged particles create charge carriers in the irradiated diamond followed by induction of an electric charge on the strips. Similarly, detonation ND and monocrystal CVD diamonds may be used for detecting both intermediate and high-energy heavy ions and in other instrumental analysis (Adam et al., 2002; Berdermann et al., 2004).

Because both carboxylated and oxidized ND exhibit a remarkably high affinity for proteins, proteins in dilute solutions can be easily captured by
NDs with a size of 100 nm, separated by centrifugation, and analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) without any need for pre-separation of the adsorbed proteins from ND (Kong et al., 2005). With the dilute mixed solution of cytochrome c, myoglobin, and albumin that preferentially adsorbs on a hydrophilic surface, the developed method offered significantly higher sensitivity than conventional MALDI-TOF-MS and had a limit of detection of 100 pM for a 1 mL sample solution. The potential of ND-assisted MALDI-TOF-MS for use in clinical proteomics was demonstrated with human blood serum analysis (Kong et al., 2005). If necessary, surfaces of ND particles can be modified to decrease non-specific protein adsorption and optimize their use for bioanalysis (Lasseter et al., 2004). Application of detonation NDs facilitated separation of Obelia longissima apoobelin from the recombinant E. coli cell extract and made possible the preparation of purified protein with a yield of up to 38% (Bondar et al., 2004).

Within the ND family, to a large extent medical applications have been developed for diamondoids. Diamondoids such as adamantane derivatives (single-molecule unit of diamond with the formula C_{10}H_{16}) have been used in pharmacology, clinical medicine, and biosensing (Freitas, 2003). Until recently the cholinesterase inhibitors were the only available drugs for the treatment of Alzheimer’s disease, which is one of the leading causes of death for people over 65 years of age. Unfortunately, the cholinesterase inhibitors cannot stop the process of neurodegeneration and just symptomatically enhance the cognitive state to some degree (Sonkusare et al., 2005). In Alzheimer’s disease, neuronal death is caused by glutamate excitotoxicity mediated through the N-methyl-D-aspartate (NMDA) receptors making them an excellent target for preventing the disease. Excitotoxicity is excessive exposure to the neurotransmitter glutamate or overstimulation of its membrane receptors, leading to neuronal injury or death. Activity of the nmDA receptor is also essential for normal neuronal function, and neuroprotective agents that fully block nmDA receptor activity will have severe side effects (Lipton, 2004). Memantine (1-amino adamantane derivative) is the potent nmDA-receptor antagonist that blocks excessive NMDA receptor activity without disrupting normal activity through its action as an uncompetitive, low-affinity, open-channel blocker. It also has beneficial effects in Parkinson’s disease, stroke, epilepsy, central nervous system (CNS) trauma, amyotrophic lateral sclerosis, drug dependence, chronic pain, depression, glaucoma, and severe neuropathic pain. Memantine is available in Europe and has been recently approved for treatment of dementia in the USA. At the present time the
second-generation memantine derivatives, which take advantage of the additional modulatory sites in the NMDA receptor that could also be used for clinical intervention, are under development (Lipton, 2004; Sonkusare et al., 2005). Many adamantane derivatives possess antibacterial, antiviral, and antifungal activity (Wang et al., 1998; El-Sherbeny, 2000; Orzeszko et al., 2002; El-Emam et al., 2004). For example, 5-(1-adamantyl)-2-substituted thio-1,3,4-oxadiazoles and 5-(1-adamantyl)-3-substituted aminomethyl-1,3,4-oxadiazoline-2-thiones exhibit substantial antimicrobial activity against Gram-positive bacteria and the antiviral activity against HIV-1 significantly reducing viral replication at 2–50 $\mu$g mL$^{-1}$ concentrations (El-Emam et al., 2004). Adamantane-based drugs, namely, amantadine and tromantadine, are excreted unaltered in the urine and are not susceptible to hydroxylation (Koppel and Tenczer, 1985). In 2005, Hodek et al. discovered that adamantane, diamantane, triamantane, 2-isopropenyl-2-methyladamantane, and 3-isopropenyl-3-methyladamantane inhibited cytochromes P450 of subfamily IIB by binding to the active site and, thus, could be potent inhibitors for hepatic oxidative drug metabolism in humans. Theoretical and experimental values of the dissociation constant of cytochrome P450 complexes with the diamondoids were in good agreement and confirmed the high potency of identified inhibitors. Bananins, the antiviral agents that possess a trioxaadamantane moiety attached to a pyridoxal derivative, were shown to be potent inhibitors of the SARS (severe acute respiratory syndrome) coronavirus helicase and can prevent replication of animal SCV (SARS-related coronavirus) (Tanner et al., 2005). Recently, the adamantane derivatives have been identified as the potential drugs acting on the P2X(7) receptor, which is involved in signaling in many inflammatory processes (cytokine release, NO generation, cytotoxicity, killing of intracellular pathogens) (Baraldi et al., 2004; Romagnoli et al., 2005).

Because ND is not mutagenic or toxic (oral LD50 value for rats is 7 g kg$^{-1}$), can neutralize free radicals, and possesses a very large surface area, it was suggested that detonation ND particles may have some anti-tumor activity (Dolmatov and Kostrova, 2000; see also Chapter 13). Indeed, mice with Erlich ascetic carcinoma that were given supplements with the ND suspensions were more active and lived almost 40% longer than non-treated animals. In 2001, Dolmatov reported the results of a new medical study of the possible oral administration of water suspensions of NDs to terminally ill cancer patients. The oral administrations of ND did not result in any side effects and, moreover, were moderately beneficial in some cases.
15.5 Biocompatibility of ND

Diamond has the outstanding reputation of a chemically inert and uniquely biologically compatible material that has found a number of applications in medicine (Freitas, 2003). Diamond is biocompatible in both bulk and particulate forms. In orthopedic surgery the use of a diamond coating on the metallic components reduces generation of macrophages and improves the wearability of devices (Santavirta et al., 1999). In addition, nanocrystalline diamond films show an excellent resistance to bacterial colonization (Jakubowski et al., 2004). The diamond-like carbon coating is also very inert and particularly suitable for use in orthopedic implants. In an early, pioneering study Thomson et al. (1991) grew mouse peritoneal macrophages and fibroblasts on tissue culture plates coated with 0.4µm of an amorphous diamond-like carbon layer and assessed the biocompatibility both biochemically and morphologically. The diamond-like carbon coating caused no adverse effects on cells in the culture. Recently diamond-like carbon films have been reexamined and it was reported that they have good biocompatibility and high corrosion resistance (Kim et al., 2005). Additional evidence of diamond biocompatibility came from Zheng et al. (2005) who fabricated nanocrystalline diamond films (NDFs) on optical glass using microwave plasma assisted CVD and used osteoblast cell cultures and platelet adhesion tests for in vitro evaluation of biocompatibility of NDFs. Their results indicated that the diamond films exhibit good tissue compatibility and hemocompatibility, which makes them very suitable for biomedical applications. The excellent chemical inertness and smoothness of NDFs made them a promising material for medical implants, cardiovascular surgery, and coating of artificial heart valves (Mitura et al., 1996; 1999). In 2004 Specht et al. were able to demonstrate the ordered growth of mammalian neurons on diamond. To accomplish this the researchers patterned proteins on diamond surfaces by micro-contact printing and cultured mouse cortical neurons on these substrates. The diamond biocompatibility and the suitability of neuron interfacing with the surface make this an interesting approach for implant engineering. The high biocompatibility, corrosion resistance, chemical inertness, low friction coefficient, electrical insulation, and excellent mechanical characteristics of CVD diamond suggested that diamond-coated materials may find numerous applications in medicine (Tang et al., 1995).

In biomaterials research, it is known that the biocompatibility of a bulk material is not necessarily the same as the biocompatibility of fine parti-
icles of the same material, which may penetrate inside live cells and their organelles (Freitas, 2003). The main threat to cell viability comes from possible mechanical damage to cellular organelles and membranes. Foreign intracellular particles with a diameter of 20–200 nm do not damage cells mechanically (Lu and Rosenzweig, 2000). Detonation particles possess a rounded shape, superior lubricity characteristics, hardness, and wear resistance. The fine diamond particles and diamond-like carbon coatings were always found to be very inert and non-inflammatory (Tse and Phelps, 1970; Hedenborg and Klockars, 1989; Swan et al., 1990; Grill, 2003). When Higson and Jones (1984) treated both pig and horse neutrophils with diamond crystals, no induction of peroxide and superoxide generation was observed. The interaction of leucocytes with diamonds with a size of 4–8 \( \mu \text{m} \) in 0.2% diamond suspension did not cause any cell damage. No increase in degranulation and production of cell motility factors, and cell death, was observed (Swan et al., 1990). Nordsletten et al. (1996) compared particles of diamond, SiC, and hydroxyapatite in serum-free cultures of human monocytes and found that all particles were phagocytozed, and monocyte morphology changed except after the ingestion of diamond. It was concluded that diamond particles were inert in a serum-free human monocyte culture, while both SiC and hydroxyapatite had a stimulatory effect comparable to that of polymethylmethacrylate. When suspensions of phagocytosable particles of diamond and SiC in hyaluronan were introduced into a canal traversing the bone implant in rabbits neither the diamond nor the SiC particles caused any decrease in bone formation. It confirmed that particles of diamond and SiC are harmless (Aspenberg et al., 1996). Human blood cells’ lack of adherence to the CVD diamond substrates, and blood clotting on diamond, produced a less rough surface than blood clotting on glass (Baranauskas et al., 2004). Recent reports on possible ND-induced damage to both white and red human blood cells in vitro (Puzyr et al., 2002; 2004b; 2005a) raised the question about the mechanism promoting these phenomena. Prior to use, NDs are usually modified to gain high stability in water colloids (Bondar and Puzyr, 2004), and it is not clear whether bacterial contamination, unidentified impurities, defects (Shames et al., 2002), and surfactants were not the factors involved. Indeed, in those studies, the total content of non-diamond carbon, non-combustible residue, and volatile compounds reached 16% in some samples (Puzyr et al., 2002). In contrast to these observations, Dion et al. (1993) did not notice any in vitro hemolysis when 14% blood solutions were treated with 0.5 g cm\(^{-3}\) diamond powder produced by De Beers Industrial Diamond Division, and the early reports on crystalline diamond-induced damage to cells were never confirmed.
Rodil et al. (2005) reported that the diamond-like coating did not have any toxic effect on human osteoblasts cells in vitro. Interestingly, Monteiro-Riviere et al. (2005) found that carbon nanotubes, which were grown using a microwave plasma enhanced CVD system, accumulated within cytoplasmic vacuoles of the human epidermal keratinocytes and stimulated release of the proinflammatory cytokine interleukin 8. There are no reports on site-specific intracellular accumulation of ND particles by live cells.

As was mentioned in the previous section, administration of detonation ND particles to both Erlich ascetic carcinoma mice and terminally ill cancer patients did not result in any side effects. Moreover, it was beneficial in some cases (Dolmatov and Kostrova, 2000; Dolmatov, 2001). However, 0.002–0.01% ND suspensions administered orally to white mice over the course of 3 months did cause a substantial increase in leukocyte content in the blood of animals (Puzyr et al., 2004c). The treatment did not affect the weight of the experimental mice though. Very unexpectedly, the intravenous administration of 0.3 mL of sterile colloids of 1% modified NDs in 10% glucose to rats and dogs did not result in sickness or premature death of the animals (Puzyr et al., 2005b). When 1 mL of 1% ND was administered to a dog followed by the second administration 1 week later, the ECG tests revealed substantial changes in heart activity immediately after each treatment. However, the cardiac condition became normal again within 1 day.

The safety and effectiveness of nanosystems and platforms made of NDs will fully depend on their compatibility with human organs, tissues, cells, and cellular organelles. Extensive thorough research has yet to be done to clearly establish physiological, immunological, and cytological responses of the human body to ND particles and films.

15.6 Conclusion

In this review, methods of surface modification were discussed for the development of functionalized diamond nanoparticles for biomedical applications. To be used in biomedical applications, nanoparticles must be biocompatible, non-toxic, non-detective by immune systems, and should not induce side effects. Size control of particles is a prerequisite for biomedical applications. To meet all these criteria, the diameter of particles should be less than 100 nm and their surfaces should be modified by hydrophilic moieties. Such nanoparticles are likely to avoid uptake by the reticuloendothelial system and remain in blood at a high enough concen-
tration to reach the target organs, tissues, or cells. The surface of nanoparticles has to be preliminarily modified by functional ligands with a high affinity to a disease site to achieve site-specific delivery. In many cases, nanoparticles may encapsulate and protect therapeutic agents against enzymes and hydrolysis; it is not clear yet how nanodiamond particles can satisfy this requirement.

It is obvious from the reviewed literature that both nanodiamond particulates and films have become very popular objects in biomedical research.

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