Luminescent nanodiamonds for biomedical applications

Jana M. Say · Caryn van Vreden · David J. Reilly ·
Louise J. Brown · James R. Rabeau ·
Nicholas J. C. King

Received: 12 August 2011 / Accepted: 13 August 2011 / Published online: 11 October 2011

© International Union for Pure and Applied Biophysics (IUPAB) and Springer 2011

Abstract In recent years, nanodiamonds have emerged from primarily an industrial and mechanical applications base, to potentially underpinning sophisticated new technologies in biomedical and quantum science. Nanodiamonds are relatively inexpensive, biocompatible, easy to surface functionalise and optically stable. This combination of physical properties are ideally suited to biological applications, including intracellular labelling and tracking, extracellular drug delivery and adsorptive detection of bioactive molecules. Here we describe some of the methods and challenges for processing nanodiamond materials,

detection schemes and some of the leading applications currently under investigation.

Keywords Nanodiamond · Nitrogen-vacancy centre · Fluorescence · Imaging · Biofunctionalisation · Drug delivery

Introduction

Nanodiamonds are typically classed as diamond particles less than 100 nm in size and are of both natural and synthetic origins. They are used in many applications, including electrochemical coatings (Dolmatov 2006; Baidakova and Vul' 2007), polymer compositions (Akopyan et al. 2004; Dolmatov 2006), polishing (Artemov 2004; Dolmatov 2006), and lubricants (Dolmatov 2006). More recently, they have been the subject of intense research in quantum optics (Kurtsiefer et al. 2000), nano-scale magnetometry (Balasubramanian et al. 2008; Maze et al. 2008) and biomedical applications that range from drug delivery to intracellular tracking (Huang et al. 2007; Pramatarova et al. 2007; Chang et al. 2008b; Liu et al. 2008; Xing and Dai 2009; Yuan et al. 2010). The biocompatibility, readily modifiable surface and optical properties originating from defects within their crystal lattice combine to make nanodiamonds extremely promising for use in biological environments. This review will focus on the characteristics, experimental progress and potential uses of a particular nanodiamond sub-class: luminescent nanodiamonds.

In the following sections we consider the key areas for successful implementation of luminescent nanodiamonds in biomedical applications, including material properties and fabrication strategies, surface modification and functional-

J. M. Say · J. R. Rabeau (✉)
ARC Centre of Excellence for Engineered Quantum Systems,
Department of Physics and Astronomy, Macquarie University,
Sydney, NSW 2109, Australia
e-mail: james.rabeau@mq.edu.au

C. van Vreden · N. J. C. King (✉)
School of Medical Sciences, Bosch Institute,
Department of Pathology, The University of Sydney,
Sydney, NSW 2006, Australia
e-mail: nickk@pathology.usyd.edu.au

D. J. Reilly
ARC Centre of Excellence for Engineered Quantum Systems,
The School of Physics, The University of Sydney,
Sydney, NSW 2006, Australia
e-mail: david.reilly@sydney.edu.au

J. M. Say · L. J. Brown
Department of Chemistry and Biomolecular Sciences,
Macquarie University,
Sydney, NSW 2109, Australia

L. J. Brown
e-mail: louise.brown@mq.edu.au

isation and behaviour and effects in a biological environment. Experimental outcomes within each of these areas will ultimately determine the suitability of nanodiamonds as a practical luminescent bio-probe. Pragmatically, there needs to be a niche in which the use of nanodiamonds, instead of conventional luminescent bio-probes, such as quantum dots or polystyrene beads, is advantageous. We will explore some of the examples where nanodiamonds have been biologically applied and the most promising areas where nanodiamonds could make an impact.

Nitrogen-vacancy centres in nanodiamond

The diverse physical and chemical properties and proposed uses of nanodiamonds depend on how they are produced. There are three major approaches to diamond synthesis: high-pressure high-temperature (HPHT), chemical vapour deposition (CVD) and detonation synthesis (DND). Nanodiamonds can also be produced by milling down larger natural or synthetic crystals. The formation of natural diamonds occurs about 200 km below the earth's surface at pressures of 70–80 kbar and temperatures of 1400–1600°C (Balmer et al. 2009). HPHT synthesis reproduces these conditions in the laboratory using large presses capable of achieving similar high pressures and temperatures in a controlled environment (Bundy et al. 1955; Field 1992). In CVD synthesis, hydrocarbon gas mixtures are ionised and, through a series of reactions, the carbon atoms are deposited onto a substrate to grow diamond films (Angus et al. 1993; Spear and Dismukes 1994; Butler and Sumant 2008; Balmer et al. 2009). DND forms in a process where carbon-containing explosives are detonated in the absence of oxygen. The explosion creates an instantaneously high temperature and pressure environment that results in the carbon being converted into diamond. The final product is always rich in graphite and other non-diamond carbon forms, which then require extensive mechanical and chemical processing to remove. The advantage of DND is the uniformly small size (4–5 nm) of the diamond grains, which is comparable to conventional fluorophores and suitable for biological applications (Danilenko 2004; Grichko and Shenderova 2006; Davydov et al. 2007).

Luminescence in nanodiamonds produced from all of these different synthesis techniques is due to the incorporation of defects in the crystal lattice. More than 500 unique optical centres have been identified in diamond (Zaitsev 2001), and several of these, including the nitrogen-vacancy (NV) (Davies and Hamer 1976; Gruber et al. 1997), silicon-vacancy (SiV) (Tomljenovic-Hanic et al. 2009), nickel-related (Gaebele et al. 2004; Rabeau et al. 2005;

Wu et al. 2006) and chromium centres (Aharonovich et al. 2010), have been targeted for applications in both quantum technologies (Prawer and Greentree 2008) and biomedical imaging (Hui et al. 2010). The NV centre is the most widely studied colour centre because of its stable photo-luminescence at room temperature and optically measurable and controllable spin states (Diep Lai et al. 2010). The remainder of this review article will focus primarily on the NV centre; however, we will also briefly discuss broader applications of nanodiamonds in biomedicine.

NV centres are atomic impurities in the diamond lattice which include a substitutional nitrogen atom adjacent to a vacancy or 'hole' in the lattice (Fig. 1a) (Davies and Hamer 1976). This vacancy enables them to carry a negative or neutral charge (Treussart et al. 2006). A number of processing techniques have been developed to artificially create the NV centre in diamond, including nitrogen implantation (Kalish et al. 1997) and electron (Dyer and Du Preez 1965) or ion (Chang et al. 2008b) irradiation. In the context of bioimaging, the most noteworthy feature, of the more abundant, negatively charged NV centre is that it can be optically excited between 480 and 580 nm to emit a broad luminescence band centred at 690 nm (Fig. 1b) with an excited state lifetime of approximately 17 ns (Fu et al. 2007).

Nanodiamonds possessing NV centres have several remarkable qualities that make them more attractive for use as probes in biomedical imaging than conventional fluorophores, such as organic fluorescent dyes and quantum dots (Table 1). The advantages of quantum dots as bioprobes are their relatively long luminescence signal and their emission wavelength, which is tuneable (Alivisatos 1996; Lidke and Arndt-Jovin 2004). However, for the imaging of medium- to long-term biological processes, quantum dot luminescence is intermittent and eventually bleaches (Lee and Osborne 2007). Moreover, quantum dots exhibit long-term cytotoxicity as their chemical components leach into the cytoplasm (Treussart et al. 2006). In comparison, nanodiamonds with NV centres are photostable, have a low toxicity to cells and also display a quantum yield close to unity. The emission wavelength of NV-containing nanodiamonds (625–800 nm) can also penetrate into tissues and is well beyond the range of cellular autofluorescence (300–500 nm) (Billinton and Knight 2001). Although a single quantum dot is threefold brighter than a nanodiamond containing a single NV centre, it is likely that with optimised fabrication, nanodiamonds will have fluorescence intensities comparable to or brighter than quantum dots (Faklaris et al. 2009a). Together, these qualities make nanodiamonds ideal for imaging long-lived biological processes.

Fig. 1 **a** Schematic of the nitrogen-vacancy (NV) centre in diamond consisting of a substitutional nitrogen atom (*N*) adjacent to a vacant lattice site (*V*) within the carbon (*C*) lattice. **b** Luminescence spectrum of an NV centre at room temperature excited by a 532-nm laser

Surface functionalisation of nanodiamond

For biological applications, it is essential to modify the surface of nanodiamonds to enable conjugation to specific molecules. Nanodiamond surfaces are typically covered in a variety of oxygen-rich functional groups, including esters, hydroxyls and carboxylic acids (Schrand et al. 2009). The process for nanodiamond functionalisation usually begins with nitric and/or sulphuric acid treatment, which removes the surface graphite and uniformly terminates the diamond surface with hydrophilic oxygen-containing groups (Ando et al. 1993). Alternatively, nanodiamonds can also be treated with reducing agents, leaving the nanodiamond surface hydrogen terminated and hydrophobic (Krüger et al. 2006; Börsch et al. 2009). Approaches used to obtain each of these terminations (Fig. 2) are shown in Table 2.

After oxidation or reduction of the nanodiamond surface, biomolecules can either be covalently or non-covalently bound. Oxidised nanodiamonds typically non-covalently adsorb bio-active molecules via hydrogen bonding, electrostatic or other polar interactions (physisorption), while hydrogen-terminated nanodiamonds are less likely to adsorb biomolecules (Steinmüller-Nethl et al. 2006; Barnard and Sternberg 2007; Krueger 2008). Although non-covalent bonding is often easier to achieve, the target moieties have a tendency to dissociate over time. In contrast, covalent bonding between the biomolecule and the nanodiamond is more stable and controllable, although it usually requires additional surface pre-activation to enable reactivity with the target molecule. A list of functional groups which have been produced on the surface of the nanodiamonds can be found in Table 3. These preparative steps have enabled a variety of

Table 1 Fluorescent properties of common fluorophores

Fluorophores	Size (nm)	Extinction coefficient ($M^{-1} cm^{-1}$)	Quantum Yield	Brightness ($M^{-1} cm^{-1}$)	Photostability	Toxicity
Nanodiamonds	4–100	8,000 (Mohan et al. 2010b)	0.99 (Mohan et al. 2010b)	7.9×10^3	Photostable, only blinks when NV centre is close to surface (Bradac et al. 2010)	Non-toxic below 400 $\mu g/mL$ (Liu et al. 2007)
Quantum dots	10–60	10^5 – 10^6 (Resch-Genger et al. 2008)	0.1–0.8 (Resch-Genger et al. 2008)	$\sim 2.5 \times 10^5$	Photostable (Lidke and Arndt-Jovin 2004) but blinks (Resch-Genger et al. 2008)	Potential toxicity from metal leakage (Treussart et al. 2006; Resch-Genger et al. 2008)
Organic dyes (e.g. Alexa Fluor, Fluorescein)	0.5–10	2.5×10^4 – 2.5×10^5 (Resch-Genger et al. 2008)	0.5–1.0 (Resch-Genger et al. 2008)	$\sim 1.0 \times 10^5$	Quickly photobleaches (Resch-Genger et al. 2008)	Dye dependant, usually not a problem (Resch-Genger et al. 2008)
Fluorescent proteins (e.g. green fluorescent protein)	10–20	30 – 92×10^3 (Kremers et al. 2011)	0.22–0.84 (Kremers et al. 2011)	$\sim 3.2 \times 10^4$	Photobleaches but slightly less quickly than organic dyes (Shaner et al. 2005)	Toxic effects are rare (Shaner et al. 2005)

Fig. 2 A nanodiamond with a mixed composition of surface groups can be oxidised and terminated with oxygen-containing functional groups or reduced to produce a homogeneous hydrogen surface termination

biologically active molecules to be non-covalently (Table 4) or covalently (Table 5) attached to nanodiamonds. For instance, poly-*L*-lysine has been both physisorbed (Huang and Chang 2004; Kong et al. 2005a; Huang et al. 2008) and covalently bonded via carbodiimide chemistry (Fu et al. 2007; Lim et al. 2009) onto the surface of acid-oxidised nanodiamonds. Other nanodiamonds have been reduced with borane, had silanes grafted to their surfaces and then were covalently bonded to biotin, a widely used universal receptor for streptavidin in biological labelling techniques (Neugart et al. 2007; Krueger et al. 2008). The wide variety of producible surface functional groups (Table 3) allows for a diverse range of bio-molecules to interact with the nanodiamond surface (Tables 4, 5).

Nanodiamond aggregation

Even after considerable surface treatments, as described above, nanodiamonds have a strong tendency to (re)aggregate in solutions and in cells, and this tendency increases with decreasing size of the nanodiamonds (Eidelman et al. 2005; Chao et al. 2007; Yuan et al. 2009). For biomedical imaging, nanodiamonds should remain separate from one another to facilitate maximal effective reaction and attachment to relevant molecules and to allow for internalisation into cells. Intracellular imaging also requires nanodiamonds of <10 nm in size. These conditions make uniform and stable de-aggregation essential. Methods currently used to de-aggregate nanodiamonds include acid treatments,

Table 2 Approaches used to oxidize and reduce nanodiamond surfaces

Oxidation/carboxylation	Reduction
Acid treatments: <ul style="list-style-type: none"> - Nitric and sulphuric acid (Kong et al. 2005a; Huang and Chang 2004; Liu et al. 2008; Chao et al. 2007; Ushizawa et al. 2002) - Nitric acid (Grichko et al. 2006; Huang et al. 2008; Sushchev et al. 2008; Mitev et al. 2007) - Hydrochloric acid (Kossovsky et al. 1995; Mitev et al. 2007) - Perchloric acid (Xu et al. 2005) - Potassium dichromate and sulphuric acid (Mitev et al. 2007) - Sulfuric acid and potassium permanganate (Xu et al. 2005) 	Hydrogen plasma treatment (Thoms et al. 1994; Strother et al. 2002; Knickerbocker et al. 2003)
Annealing in: <ul style="list-style-type: none"> - Ozone (Petrov et al. 2007; Krüger et al. 2006) - Air (Mochalin and Gogotsi 2009; Osswald et al. 2006; Shenderova et al. 2006; John et al. 2003; Mitev et al. 2007) 	Annealing in hydrogen gas (Härtl et al. 2004; Christiaens et al. 2006; Saini et al. 2008)
	Water vapour treatment (Ando et al. 1996a)
	Borane (BH₃) (Krüger et al. 2006; Neugart et al. 2007; Liang et al. 2009; Zhang et al. 2009b)
	Lithium Aluminum hydride (Hens et al. 2008; Zheng et al. 2009; Liu et al. 2010)

Table 3 Functional groups which have been produced on nanodiamonds

Amine (Miller and Brown 1996; Knickerbocker et al. 2003; Lu et al. 2004; Strother et al. 2002; Yang et al. 2002; Takahashi et al. 2003; Yeap et al. 2008; Zhang et al. 2009b; Zheng et al. 2009; Chang et al. 2010)	Carboxyl (Strother et al. 2002; Takahashi et al. 2003; Ida et al. 2003; Tsubota et al. 2003; Härtl et al. 2004; Christiaens et al. 2006; Chang et al. 2010)	Fluorine (Liu et al. 2004; Ando et al. 1996b; Khabashesku et al. 2005; Ando et al. 1996a; Ando et al. 1993)	Chlorine (Ando et al. 1996a; Ikeda et al. 1998; Takahashi et al. 2003; Miller and Brown 1996)
Silanes (Zhang et al. 2009b; Liang et al. 2009; Krüger et al. 2006; Yeap et al. 2008)	Aryl (Liang et al. 2009; Mangeney et al. 2008; Yeap et al. 2009)	Alkyl (Liu et al. 2004; Krueger and Boedeker 2008; Chang et al. 2010)	Amino (Liu et al. 2004; Ando et al. 1996a; Härtl et al. 2004; Khabashesku et al. 2005)
Butyl (Khabashesku et al. 2005; Ikeda et al. 1998)	Azide (Barras et al. 2010; Zheng et al. 2009)	Methyl (Khabashesku et al. 2005)	Glycine (Khabashesku et al. 2005)
Hexyl (Khabashesku et al. 2005)	Benzoquinone (Purtov et al. 2010)	Perfluorooctyl ester (Nakamura et al. 2003)	Ether (Nakamura et al. 2003)
Amino acid (Liu et al. 2004)	Thymidine (Ushizawa et al. 2002)	Aliphatic (Chang et al. 2010)	Phenyl (Chang et al. 2010)
	Cyanide (Zheng et al. 2009)	Chlorocarbonyl (Ushizawa et al. 2002)	Halides (Zheng et al. 2009)
			Thiols (Zheng et al. 2009)

bead milling and sonication (Krüger et al. 2005; Fu et al. 2007; Faklaris et al. 2008). Through a combination of these techniques we were first able to observe NV centres in isolated 5-nm nanodiamonds (Bradac et al. 2010). However, in order to achieve long-term stability, the repulsive forces between the particles must be increased. This can be done by atmospheric plasma treatment with

nitrogen or hydrogen (Gibson et al. 2009), annealing in hydrogen gas (Williams et al. 2010) or treatment with NaCl (Bondar' and Puzyr' 2004) or sodium oleate (Xu et al. 2005). Functionalisation with some biomolecules, such as poly-*L*-lysine (Vaijayanthimala et al. 2009) and bovine serum albumin (Yuan et al. 2009), can also increase nanodiamond solubility and reduce re-aggregation. The

Table 4 Various biological moieties which have been physisorbed to nanodiamonds

Proteins/Enzymes	Toxins	Other:
- apoobelin (Bondar' et al. 2004)	- aflatoxin B1 (Afb1) (Puzyr' et al. 2007)	- DNA (Fu et al. 2007)
- luciferase (Bondar' et al. 2004; Puzyr' et al. 2004)	- Alpha-bungarotoxin (Liu et al. 2008)	- Cellobiose (Kossovsky et al. 1995)
- bone morphogenetic protein-2 (BMP-2) (Steinmüller-Nethl et al. 2006)	Drugs	- Glass-like carbon (Guan et al. 2006)
- catalase (Puzyr et al. 2007)	- doxorubicin (DXR) (Burlison et al. 2009)	- Poly- <i>L</i> -lysine (Huang et al. 2008; Huang and Chang 2004; Kong et al. 2005a)
- histone (Puzyr et al. 2007)	- paclitaxel (PTX) (Burlison et al. 2009)	- 800 Da polyethyleneimine (PEI800) (Zhang et al. 2009b)
- lysozyme (Puzyr et al. 2007; Chao et al. 2007; Chung et al. 2006; Nguyen et al. 2007; Liu and Sun 2010)	- doxorubicin hydrochloride (DOX) (Huang et al. 2007, 2008)	- Sodium dodecyl sulfate (SDS) (Neugart et al. 2007)
- obelin (Purtov et al. 2001)	- dexamethasone (DEX) (Huang et al. 2008; Chen et al. 2009)	- Sodium oleate (Xu et al. 2005)
- horse cytochrome <i>c</i> (HCC) (Huang and Chang 2004; Kong et al. 2005b; Nguyen et al. 2007)	- Purvalanol A (Chen et al. 2009)	- Polyallylamine (PAAm) (Saini et al. 2008)
- horse heart myoglobin (Mb) (Kong et al. 2005b)	- 4-hydroxytamoxifen (Chen et al. 2009)	- Rhodamine (Zhang et al. 2009b)
- bovine serum albumin (BSA) (Kong et al. 2005b; Yeap et al. 2008)	Antibodies	
- ovalbumin (Yeap et al. 2008)	- Mouse immunoglobulin G (IgG) (Purtov et al. 2010)	
- fetuin (Yeap et al. 2008)	Hormones	
- ribonuclease B (Yeap et al. 2008)	- bovine insulin (Shimkunas et al. 2009)	
- cholesterol esterase (Puzyr et al. 2007)		
- cholesterol oxidase (Puzyr et al. 2007)		
- peroxidase (Puzyr et al. 2007)		

Table 5 Various biological moieties which have been covalently bonded to nanodiamonds via amide bonds unless otherwise stated

Proteins/Enzymes	Hormones	Other cont.
- transferrin (Weng et al. 2009)	- growth hormone (Cheng et al. 2007)	- NHS-Biotin (Hens et al. 2008)
- green fluorescent protein (GFP) (Härtl et al. 2004)	Antibodies against	- Biotin (Krueger et al. 2008; Neugart et al. 2007) (silane bond)
- bovine serum albumin (BSA) (Mohan et al. 2010a; Purtov et al. 2010)	- Mitochondria (Mkandawire et al. 2009)	- Poly-L-lysine (Fu et al. 2007; Lim et al. 2009; Mohan et al. 2010a; Vijayanthimala et al. 2009)
- Porcine trypsin (Wei et al. 2010)	- Actin (Mkandawire et al. 2009)	- Polyethylene glycol (PEG)- diamine (Zhang et al. 2009a)
- asparagine amidase F (PNGase F) (Wei et al. 2010)	- Rabbit anti-mouse (Purtov et al. 2010)	- Folic acid (Zhang et al. 2009a)
Dye molecules	- Mouse immunoglobulin G (IgG) (Purtov et al. 2010)	- Amino acids (Krüger et al. 2006; Krueger et al. 2007) (Silane bond)
- octadecylamine (ODA) (Mochalin and Gogotsi 2009)	Drugs	- Silicon chip (Liu and Sun 2010)
- NHS-TAMRA (Hens et al. 2008)	- Paclitaxel (PTX) (Liu et al. 2010)	- Dextran (Mohan et al. 2010a)
- IRDye-800CW (Mohan et al. 2010b)	Other	
	- DNA (Christiaens et al. 2006; Takahashi et al. 2003; Knickerbocker et al. 2003; Lu et al. 2004; Rezek et al. 2006; Yang et al. 2002)	
	- DNA (Ushizawa et al. 2002) (ester linkage)	

choice of biological buffer systems or media is also important, since both foetal bovine serum and phosphate buffered saline, two common biological buffer systems, tend to cause re-aggregation (Neugart et al. 2007; Vijayanthimala et al. 2009). Despite the challenges involved in preparing and maintaining de-aggregated nanodiamonds, positive results strongly support their use in biological applications.

Bio-compatibility of nanodiamond

The stability and innate biocompatibility of carbon in nanodiamond significantly mitigates biochemical interactions within the host cell. The measurement of mitochondrial damage using the methyl thiazolyl tetrazolium assay revealed that the toxicity of nanodiamonds (Schrand et al. 2009) in a wide range of cell lines is extremely low (Table 6). In acute conditions, cells tolerate concentrations up to about 400 µg/mL of raw or surface functionalised nanodiamonds in vitro (Yu et al. 2005; Chao et al. 2007; Liu et al. 2007; Schrand et al. 2007; Faklaris et al. 2008; Vijayanthimala et al. 2009), with a decrease by approximately 20% in cell viability in some cancer cells at concentrations of 4×10^7 nanodiamonds/mL (Burleson et al. 2009). Protein expression (Liu et al. 2007), rates of cellular proliferation and differentiation (Vijayanthimala et al. 2009), as well as rates of apoptosis (Liu et al. 2007) have all been used to show nanodiamond in vitro biocompatibility. This is further borne out by the low levels of reactive oxygen species measurable in treated cells since high levels of reactive oxygen species are a common sign of cell stress (Schrand et al. 2007). In

chronic conditions, there has also been little or no discernable difference between treated lung cancer and embryonic fibroblast cells and control cells (Liu et al. 2009). Thus, in contrast to other nanoparticle labels, such as quantum dots, which release biologically toxic substances over time (Treussart et al. 2006), there is good evidence for the biocompatibility of nanodiamond-based labeling schemes in vitro.

In contrast to in vitro labeling, in vivo tests have shown a higher degree of toxicity. Nanodiamond exposure has been tested by topical application to the skin and intratracheal administration. In one study, the application of nanodiamonds to the skin of mice did not cause contact hypersensitivity or other allergic reactions (Burleson et al. 2009). At a dose of 1mg/kg,

Table 6 Cells showing good biocompatibility with nanodiamond in vitro

HeLa cells (human cervical carcinoma) (Faklaris et al. 2008; Vijayanthimala et al. 2009)
Human lung adenocarcinoma cells (Chao et al. 2007; Liu et al. 2007; Liu et al. 2009)
Keratinocytes (Burleson et al. 2009; Schrand et al. 2007)
Neuroblastomas (Schrand et al. 2007)
PC-12 cells (Schrand et al. 2007)
Lung fibroblasts (Liu et al. 2007)
Embryonic fibroblasts (Liu et al. 2009; Vijayanthimala et al. 2009)
Human kidney cells (Yu et al. 2005)
Osteoprogenitor cells (Vijayanthimala et al. 2009)
Macrophages (Schrand et al. 2007)

4 nm nanodiamonds instilled into the mouse trachea were phagocytosed by alveolar macrophages within 24 h, with a decrease in the number of nanodiamonds observed in the alveolar area throughout the study. Histopathological and ultrastructural investigations at 7, 14 and 28 days post-exposure also showed no adverse effects in the lungs; nor did nanodiamonds evidently translocate into the bloodstream and/or other organs (Yuan et al. 2010). However, in another study utilising significantly higher doses administered intratracheally (4 and 20 mg/kg), the mice showed some signs of toxicity over a shorter 3 day timeframe (Zhang et al. 2010).

The bio-toxicity of nanodiamond via more invasive routes, such as injection beneath the skin (subcutaneously), injection into the lining of the abdominal cavity (intraperitoneally) and injection into the blood (intravenously), have also been studied. Subcutaneous (Puzyr et al. 2007) and intraperitoneal (Mitura et al. 2001; Bakowicz and Mitura 2002) injections into mice and rats, respectively, showed that aggregates of nanodiamonds could be found adjacent to undamaged cells (Puzyr et al. 2007) and that there was little local inflammatory response (Mitura et al. 2001; Bakowicz and Mitura 2002). The mice also showed no signs of weight-loss or other clinical signs of toxicity even 28 days after the intravenous injection of nanodiamonds (Yuan et al. 2009). However, intravenous injection of nanodiamonds into rabbits showed a short-term increase in serum bilirubin, suggesting an increase in red blood cell lysis, even though red blood cell numbers and haemoglobin concentration remained constant. A number of other changes were also observed, such as decreased serum cholesterol and low-density lipoproteins and increased triglycerides, suggesting metabolic changes, probably associated with the sequestering of the nanodiamonds in the liver (Puzyr et al. 2007).

Since the accumulation of intravenously injected nanodiamonds has important implications for applications such as drug delivery, their distribution within the body was studied with I^{125} -conjugated nanodiamonds (Yuan et al. 2009). The nanodiamonds were found to be rapidly sequestered into the liver (approx. 40%) with the lungs (approx. 6%) and spleen (approx. 1%) exhibiting significantly lower uptake. Tests 28 days after injection showed that approximately 60% of the nanodiamonds were concentrated in the liver with less than 10% in the lungs (Yuan et al. 2009). These results suggest that the reticuloendothelial system plays a major role in the uptake (i.e. clearance) of nanodiamonds, as it does for other particulate matter in the blood. However, this uptake may well vary, depending on what is conjugated to the nanodiamonds. In terms of eventual loading, the importance of this pattern of uptake has functional implications for these organs as well as for individual cells.

Much research has focussed on the cellular interactions of nanodiamonds and their mechanisms of internalisation. Optical measurements based on luminescence (Yu et al. 2005; Chao et al. 2007; Chang et al. 2008a, b; Faklaris et al. 2008, 2009b; Liu et al. 2009; Yuan et al. 2009; Zhang et al. 2009a) or Raman signatures (Cheng et al. 2007; Chao et al. 2007) have shown that nanodiamonds are internalised and tend to be located in endocytic vesicles, but they do not enter the nucleus. This has been confirmed by transmission electron microscopy and atomic force microscopy (Chang et al. 2008a, b; Faklaris et al. 2008, 2009b; Yu et al. 2005; Liu et al. 2009; Zhang et al. 2009a).

Various pathways of cellular internalisation have been suggested (Nordsletten et al. 1996; Chang et al. 2008a; Liu et al. 2009). Studies with 25–30 nm nanodiamonds have shown that internalisation is predominantly receptor-mediated and clathrin-dependent (Faklaris et al. 2009b; Vijayanthimala et al. 2009; Zhang et al. 2009a). Thus, treating cells with sodium azide and 2-deoxyglucose, which reduces available energy for internalisation, or with phenyl arsine oxide, a clathrin-specific inhibitor, significantly reduces the internalisation of 140 nm nanodiamonds; in contrast, filipin, a caveolin-specific inhibitor does not. This result is further supported by clathrin staining during endocytosis (Liu et al. 2009). However, nanodiamond size, conjugated functional groups, solution and cell type all influence the process. For example, Weng et al. (2009) found that transferrin-conjugated 100-nm diamonds were taken up via the abundant transferrin receptors on HeLa cells in a clathrin-dependant manner. In contrast, Cheng et al. (2007) observed that similar-sized nanodiamonds conjugated to growth hormone remained extracellular, presumably attached to the binding domain of the growth hormone receptor on A549 cells, while the unconjugated control nanodiamonds were internalised. Nanodiamonds can also be internalised by other receptor-mediated pathways. For instance, nanodiamonds conjugated to folate are internalised by caveolin-dependent endocytosis (Zhang et al. 2009a). The possible internalisation pathways, distribution within the body and biocompatibility in vitro and in vivo make nanodiamonds promising bio-probes.

Nanodiamond applications: Imaging, tracking and molecular carriers

The biocompatibility of nanodiamonds has made it possible to use them in a multitude of possible applications in biomedicine. Their readily modifiable surface allows designated attachment of the widest possible variety of biologically interactive molecules, and their physical properties enable them to be detected by a wide range

of modalities. As a consequence, a range of multimodal detection schemes are being recognised whereby the optical or spin signatures can be detected and tracked both *in vivo* and *in vitro*. In addition to a growing interest in the use of nanodiamonds to investigate and track physiological processes within cells, there is considerable interest in the use of nanodiamonds for targeted delivery of biological payloads to abrogate or modulate cell function.

Detection, imaging and tracking *in vitro*

The combination of images and signals produced by bright field and wide field epifluorescence has enabled the reconstruction of three-dimensional images and trajectories of nanodiamonds (Fig. 3) (Fu et al. 2007; Chang et al. 2008b; Zhang et al. 2009a) more recently in real time (Faklaris et al. 2009a) using single particle tracking within cells. As nanodiamonds do not enter the nucleus (Yu et al. 2005; Chang et al. 2008a, b; Wee et al. 2009; Zhang et al. 2009a) and because their bright fluorescence is emitted beyond the range of cellular autofluorescence (Yu et al. 2005; Fu et al. 2007; Fucikova et al. 2009; Weng et al. 2009), they can be used as cytoskeletal markers at concentrations as low as 1 $\mu\text{g/mL}$ and detected using confocal fluorescence microscopy (Chao et al. 2007). Furthermore, because of the long radiative lifetime of NV colour centres (approx. 17 ns) (Fu et al. 2007) relative to cell auto-fluorescence, time-gated confocal imaging can also be used to enhance the nanodiamond signal (Faklaris et al. 2008). Two-photon excitation microscopy can further improve resolution and contrast and minimise background noise (Chang et al. 2008b).

Alternatively, although giving much less architectural information, accurate quantitative studies have been performed using fluorescence-based cytometry. In these studies, nanodiamonds were internalised by or attached to cells via a specific molecule of interest. The quantitative information was then gathered from the emitted fluores-

cence of the cell population in a FACS-Array or from the individual cells in a flow cytometer (Liu et al. 2008; Liu et al. 2010; Li and Zhou 2010). These approaches are important when the aim is to gather data from large numbers of cells, and they have been successfully used with fluorescent polystyrene beads for tracking macrophage lineage (i.e. using their phagocytic function) cells *in vivo* (Getts et al. 2008).

In vivo magnetic resonance imaging with nanodiamond-based contrast agents

Magnetic resonance imaging (MRI) provides *in vivo* accessibility to physiological interactions of central importance in the detection and treatment of a wide range of diseases (Blamire 2008). The use of nanodiamonds as MRI contrast agents has the potential to enable the monitoring of cellular events in intact tissue with a degree of specificity not provided by conventional MRI and is essential for the visualisation of particular cellular processes, such as receptor binding. Current targeted contrast agents go some of the way to meeting this challenge, with potential to track specific cells or receptors *in vivo* (Weissleder 1999). However the sensitivity is inherently low and the contrast agents (Weissleder 1991) can be toxic.

One approach to achieving MRI contrast using nanodiamond is to attach a magnetic species, such as Gd(III) complexes, to the nanodiamond surface (Manus et al. 2010). The presence of a paramagnetic metal shortens the longitudinal spin relaxation time (T_1) of water protons in the surrounding tissue and leads to contrast in T_1 -weighted imaging (Caravan et al. 1999). This technique has recently been demonstrated to enhance MRI contrast through covalent modification of the nanodiamond surface to allow the conjugation of Gd(III) complexes (Manus et al. 2010).

A second approach is to directly detect the ^{13}C nuclear spins in the nanodiamond itself. The inherent difficulty with this technique is the low sensitivity of nuclear

Fig. 3 **a)** Combined bright-field and epifluorescence images of nanodiamonds in a live HeLa **b)** A reconstruction of the cell (left) and three-dimensional tracking of a single 35 nm nanodiamond (the

yellow-boxed nanodiamond in part **a)** over a period of 200 s (right). Reprinted by permission from Macmillan Publishers Ltd: Nature Nanotechnology (Chang et al. 2008b), copyright 2008

magnetism in combination with the low natural abundance of spin-active ^{13}C (1.1%) relative to ^{12}C . Methods to overcome this limitation include the production of nanodiamond grown from ^{13}C -rich sources and the use of nuclear hyperpolarisation (Hoch and Reynhardt 1988; Reynhardt and Terblanche 1997) which can dramatically increase the nuclear resonance signal.

Nanodiamonds as molecular carriers

The targeted delivery of therapeutic DNA (Zhang et al. 2009b) or cytotoxic drugs (Huang et al. 2007; Liu et al. 2010) could maximise the efficacy of therapy as well as minimise the side effects and/or collateral damage to normal tissue *in vivo*. Nanodiamonds can potentially provide a delivery vehicle for these treatments. For example, doxorubicin (DOX) and paclitaxel (PTX), two cytotoxic drugs widely used in cancer chemotherapy, have been successfully and stably absorbed onto nanodiamonds (Huang et al. 2007; Burleson et al. 2009; Liu et al. 2010). The more insoluble purvalanol A, and 4-hydroxytamoxifen, used more specifically in liver and breast cancer, respectively, have also been absorbed onto nanodiamonds while retaining their cytotoxicity (Chen et al. 2009). Ironically, the undesirable tendency of nanodiamonds to form aggregates may provide a basis for more effective treatment *in vivo*, as the surface-adsorbed drug is initially released, followed by a more gradual and constant release of the drug from within the aggregates (Huang et al. 2007). If successful, this strategy would reduce the need for systemic drug administration and produce a more sustained local exposure, potentially decreasing damage to remote healthy cells and reducing consequential clinical side-effects.

In itself, however, conjugating a drug to a nanodiamond does not produce targeted delivery. Recently, stable covalent conjugation of both I^{125} -labelled bovine serum albumin and rabbit anti-mouse immunoglobulin G was reported with the antibody retaining its ability to bind to its cognate antigen (Purtov et al. 2010). This result opens the door for specifically targeted delivery of a biological payload while simultaneously enabling tumour imaging *in vivo* via the local accumulation of luminescent nanodiamonds at the tumour site. As such, it represents a valuable means for optimising the efficacy of drugs and drug delivery. Such specific targeting would also enable the use of reduced amounts of highly toxic agents that would otherwise not achieve high enough local concentrations to be effective without catastrophic side-effects if given to patients systemically. This approach, while obvious for cancer, could easily be adapted for various infectious diseases, particularly infections by extracellular organisms, those with extracellular phases, such as malaria, and/or those expressing pathogen-specific molecules on infected

cell surfaces. Furthermore, specific cellular subsets in diseases characterised by over-exuberant pro-inflammatory immune responses could be targeted and inactivated or destroyed, for example, in autoimmune disease and even certain infectious diseases (Getts et al. 2008; Getts et al. 2011). Dually conjugated nanodiamonds could also be used to target cell subsets expressing a particular pro-inflammatory chemokine or cytokine receptor using the relevant chemokine or cytokine to deliver a behaviour-modifying signal to these cells (Smith et al. 2011). Such signals could be used to block migration or inhibit the pro-inflammatory response of the targeted cells.

Conclusions

The biomedical application of nanodiamonds in experimentation, diagnostics, drug delivery and therapeutics relies on the size, inertness, stability, fluorescence and a host of manipulable surface characteristics. The recent interest in this field has been driven by the promising results thus far obtained, which have further amplified interest in nanodiamonds. While still in its infancy, the use of nanodiamond holds enormous promise for biomedical diagnostics and therapeutics for both humans and animals.

Acknowledgements We acknowledge Carlo Bradac for his artistic rendering of an NV centre. We would also like to acknowledge the support from the Australian Research Council Future Fellowship scheme (FT0991243), Discovery Projects scheme (DP1094439) and Centres of Excellence (CE110001013). JMS is also supported by an International Macquarie Research Excellence Scholarship and NJCK acknowledges support from the National Health and Medical Research Council Project Grant 512413.

Ethical Standards The authors would like to acknowledge that since this is a review and as such does not present any unpublished experimental work by our group that the ethical standards of Australia were upheld.

Conflict of Interest The authors declare that they have no conflict of interest.

References

- Aharonovich I, Castelletto S, Johnson BC, McCallum JC, Simpson DA, Greentree AD, Prawer S (2010) Chromium single-photon emitters in diamond fabricated by ion implantation. *Phys Rev B* 81:121201. doi:10.1103/PhysRevB.81.121201
- Akopyan LA, Zlotnikov MN, Rumyantsev BV, Abramova NL, Zobina MV, Mordvintseva TL (2004) Synthesis of explosive decompression-resistant rubbers with the use of detonation carbon. *Phys Solid State* 46(4):742–745. doi:10.1134/1.1711463

- Alivisatos AP (1996) Semiconductor clusters, nanocrystals, and quantum dots. *Science* 271(5251):933–937. doi:10.1126/science.271.5251.933
- Ando T, Tanaka J, Ishii M, Kamo M, Sato Y, Ohashi N, Shimozaki S (1993) Diffuse reflectance Fourier-transform infrared study of the plasma-fluorination of diamond surfaces using a microwave discharge in CF₄. *J Chem Soc, Faraday Transactions* 89 (16):3105–3109. doi:10.1039/f9938903105
- Ando T, Nishitani-Gamo M, Rawles RE, Yamamoto K, Kamo M, Sato Y (1996a) Chemical modification of diamond surfaces using a chlorinated surface as an intermediate state. *Diam Relat Mater* 5:1136–1142. doi:10.1016/0925-9635(96)00529-8
- Ando T, Yamamoto K, Matsuzawa M, Takamatsu Y, Kawasaki S, Okino F, Touhara H, Kamo M, Sato Y (1996b) Direct interaction of elemental fluorine with diamond surfaces. *Diam Relat Mater* 5:1021–1025. doi:10.1016/0925-9635(96)00521-3
- Angus JC, Argoitia A, Gat R, Li Z, Sunkara M, Wang L, Wang Y (1993) Chemical vapour deposition of diamond. *Phil Trans Roy Soc A* 342(1664):195–208. doi:10.1098/rsta.1993.0014
- Artemov AS (2004) Polishing nanodiamonds. *Phys Solid State* 46 (4):687–695. doi:10.1134/1.1711463
- Baidakova M, Vul' A (2007) New prospects and frontiers of nanodiamond clusters. *J Phys D: Appl Phys* 40:6300–6311. doi:10.1088/0022-3727/40/20/S14
- Bakowicz K, Mitura S (2002) Biocompatibility of NCD. *J Wide Bandgap Mater* 9(4):261–272. doi:10.1106/152451102024429
- Balasubramanian G, Chan IY, Kolesov R, Al-Hmoud M, Tisler J, Shin C, Kim C, Wojcik A, Hemmer PR, Krueger A, Hanke T, Leitenstorfer A, Bratschitsch R, Jelezko F, Wrachtrup J (2008) Nanoscale imaging magnetometry with diamond spins under ambient conditions. *Nature* 455:648–651. doi:10.1038/nature07278
- Balmer RS, Brandon JR, Clewes SL, Dhillon HK, Dodson JM, Friel I, Inglis PN, Madgwick TD, Markham ML, Mollart TP, Perkins N, Scarsbrook GA, Twitchen DJ, Whitehead AJ, Wilman JJ, Woollard SM (2009) Chemical vapour deposition synthetic diamond: materials, technology and applications. *J Phys: Condens Matter* 21:364221. doi:10.1088/0953-8984/21/36/364221
- Barnard AS, Sternberg M (2007) Crystallinity and surface electrostatics of diamond nanocrystals. *J Mater Chem* 17:4811–4819. doi:10.1039/b710189a
- Barras A, Szunerits S, Marcon L, Monfilliet-Dupont N, Boukherroub R (2010) Functionalization of diamond nanoparticles using “click” chemistry. *Langmuir* 26(16):13168–13172. doi:10.1021/la101709q
- Billinton N, Knight AW (2001) Seeing the wood through the trees: a review of techniques for distinguishing green fluorescent protein from endogenous autofluorescence. *Anal Biochem* 291:175–197. doi:10.1006/abio.2000.5006
- Blamire AM (2008) The technology of MRI—the next 10 years? *Br J Radiol* 81:601–617. doi:10.1259/bjr/96872829
- Bondar' VS, Puzyr' AP (2004) Nanodiamonds for biological investigations. *Phys Solid State* 46(4):716–719. doi:10.1134/1.1711457
- Bondar' VS, Pozdnyakova IO, Puzyr' AP (2004) Applications of nanodiamonds for separation and purification of proteins. *Phys Solid State* 46(4):758–760. doi:10.1134/1.1711468
- Börsch M, Reuter R, Balasubramanian G, Erdmann R, Jelezko F, Wrachtrup J (2009) Fluorescent nanodiamonds for FRET-based monitoring of a single biological nanomotor F₀F₁-ATP synthase. In: *Pro SPIE*, p 71832. doi:10.1117/12.812720
- Bradac C, Gaebel T, Naidoo N, Sellars MJ, Twamley J, Brown LJ, Barnard AS, Plakhotnik T, Zvyagin AV, Rabeau JR (2010) Observation and control of blinking nitrogen-vacancy centres in discrete nanodiamonds. *Nat Nanotechnol* 5:345–349. doi:10.1038/nnano.2010.56
- Bundy FP, Hall HT, Strong HM, Wentorf RHJ (1955) Man-made diamonds. *Nature* 176(4471):51–55. doi:10.1038/176051a0
- Burleson T, Yusuf N, Stanishevsky A (2009) Surface modification of nanodiamonds for biomedical application and analysis by infrared spectroscopy. *J Ach Mat Manufac Eng* 37(2):258–263
- Butler JE, Sumant AV (2008) The CVD of nanodiamond materials. *Chem Vap Depos* 14:145–160. doi:10.1002/cvde.200700037
- Caravan P, Ellison JJ, McMurry TJ, Lauffer RB (1999) Gadolinium (III) chelates as MRI contrast agents: structure, dynamics, and applications. *Chem Rev* 99:2293–2352. doi:10.1002/chin.199947322
- Chang IP, Hwang KC, Chiang C-S (2008a) Preparation of fluorescent magnetic nanodiamonds and cellular imaging. *J Am Chem Soc* 130:15476–15481. doi:10.1021/ja804253y
- Chang Y-R, Lee H-Y, Chen K, Chang C-C, Tsai D-S, Fu C-C, Lim T-S, Tzeng Y-K, Fang C-Y, Han C-C, Chang H-C, Fann W (2008b) Mass production and dynamic imaging of fluorescent nanodiamonds. *Nat Nanotechnol* 3:284–288. doi:10.1038/nnano.2008.99
- Chang IP, Hwang KC, J-aA H, Lin C-C, Hwu RJ-R, Horng J-C (2010) Facile surface functionalization of nanodiamonds. *Langmuir* 26 (5):3685–3689. doi:10.1021/la903162v
- Chao J-I, Perevedentseva E, Chung P-H, Liu K-K, Cheng C-Y, Chang C-C, Cheng C-L (2007) Nanometer-sized diamond particle as a probe for biolabeling. *Biophys J* 93:2199–2208. doi:10.1088/0957-4484/19/20/205102
- Chen M, Pierstorff ED, Lam R, Li S-Y, Huang H, Osawa E, Ho D (2009) Nanodiamond-mediated delivery of water-insoluble therapeutics. *ACS Nano* 3(7):2016–2022
- Cheng C-Y, Perevedentseva E, Tu J-S, Chung P-H, Cheng C-L, Liu K-K, Chao J-I, Chen P-H, Chang C-C (2007) Direct and in vitro observation of growth hormone receptor molecules in A549 human lung epithelial cells by nanodiamond labeling. *Appl Phys Lett* 90:163903. doi:10.1063/1.2727557
- Christians P, Vermeeren V, Wenmackers S, Daenen M, Haenen K, Nesládek M, vandeVen M, Ameloot M, Michiels L, Wagner P (2006) EDC-mediated DNA attachment to nanocrystalline CVD diamond films. *Biosens Bioelectron* 22:170–177. doi:10.1016/j.bios.2005.12.013
- Chung P-H, Perevedentseva E, Tu J-S, Chang CC, Cheng C-L (2006) Spectroscopic study of bio-functionalized nanodiamonds. *Diam Relat Mater* 15:622–625. doi:10.1016/j.diamond.2005.11.019
- Danilenko VV (2004) On the history of the discovery of nanodiamond synthesis. *Physics of the Solid State* 46(4):595–599. doi:10.1134/1.1711431
- Davies G, Hamer MF (1976) Optical studies of the 1.945 eV vibronic band in diamond. *Proc Roy Soc Lond Ser A* 348(1653):285–298
- Davydov VA, Rakhmanina AV, Rols S, Agafonov V, Pulikkathara MX, Vander Wal RL, Khabashesku VN (2007) Size-dependent phase transition of diamond to graphite at high pressures. *J Phys Chem C* 111:12918–12925. doi:10.1021/jp073576k
- Diep Lai N, Zheng D, Treussart F, Roch J-F (2010) Optical determination and magnetic manipulation of a single nitrogen-vacancy color center in diamond nanocrystal. *Adv Natl Sci: Nanosci Nanotechnol* 1:015014. doi:10.1088/2043-6254/1/1/015014
- Dolmatov VY (2006) Applications of Detonation Nanodiamond. In: Shenderova OA, Gruen DM (eds) *Ultrananocrystalline Diamond: Synthesis, Properties, and Applications*. William Andrew Publ, Norwich New York, pp 477–527
- Dyer HB, Du Preez L (1965) Irradiation damage in type I diamond. *J Chem Phys* 42(6):1898–1906. doi:10.1063/1.1696224
- Eidelman ED, Siklitsky VI, Sharonova LV, Yagovkina MA, Vul AY, Takahashi M, Inakuma M, Ozawa M, Ōsawa E (2005) A stable suspension of single ultrananocrystalline diamond particles. *Diam Relat Mater* 14:1765–1769. doi:10.1016/j.diamond.2005.08.057
- Faklaris O, Garrot D, Joshi V, Druon F, Boudou J-P, Sauvage T, Georges P, Curmi PA, Treussart F (2008) Detection of single

- photoluminescent diamond nanoparticles in cells and study of the internalization pathway. *Small* 4(12):2236–2239. doi:10.1002/sml.200800655
- Faklaris O, Garrot D, Joshi V, Boudou J-P, Sauvage T, Curmi PA, Treussart F (2009a) Comparison of the photoluminescence properties of semiconductor quantum dots and non-blinking diamond nanoparticles. Observation of the diffusion of diamond nanoparticles in living cells. *J Eur Optical Soc: Rapid Publ* 4:090325. doi:10.2971/jeos.2009.09035
- Faklaris O, Joshi V, Irinopoulou T, Tauc P, Girard H, Gesset C, Senour M, Thorel A, Arnault J-C, Boudou J-P, A. Curmi P, Treussart F (2009b) Determination of the internalization pathway of photoluminescent nanodiamonds in mammalian cells for biological labeling and optimization of the fluorescent yield. arXivorg, e-Print Archive: arXiv:0907.1148v0901 [physics.optics]. Available at: <http://hal.archives-ouvertes.fr/docs/00/40/17/98/PDF/nanodiamonds.pdf>
- Field JE (1992) The properties of natural and synthetic diamond. Academic Press, London
- Fu C-C, Lee H-Y, Chen K, Lim T-S, Wu H-Y, Lin P-K, Wei P-K, Tsao P-H, Chang H-C, Fann W (2007) Characterization and application of single fluorescent nanodiamonds as cellular biomarkers. *Proc Natl Acad Sci USA* 104(3):727–732. doi:10.1073/pnas.0605409104
- Fucikova A, Valenta J, Pelant I, Brezina V (2009) Novel use of silicon nanocrystals and nanodiamonds in biology. *Chem Pap* 63 (6):704–708. doi:10.2478/s11696-009-0075-x
- Gaebel T, Popa I, Gruber A, Domhan M, Jelezko F, Wrachtrup J (2004) Stable single-photon source in the near infrared. *New J Phys* 6:98. doi:10.1088/1367-2630/6/1/098
- Getts DR, Terry RL, Getts MT, Müller M, Rana S, Shrestha B, Radford J, Van Rooijen N, Campbell IL, King NJC (2008) Ly6c + "inflammatory monocytes" are microglial precursors recruited in a pathogenic manner in West Nile virus encephalitis. *The J Exp Med* 205:2319–2337. doi:10.1084/jem.20080421
- Getts D, Turley D, Smith C, Harp C, McCarthy D, Feeney E, Getts M, Martin A, Luo X, Terry R, King N, Miller S (2011) Tolerance induced by apoptotic antigen-coupled leukocytes is induced by PD-L1+, IL-10-producing splenic macrophages and maintained by tregs. *J Immunol* 187(5) (in press)
- Gibson N, Shenderova O, Luo TJM, Moseenkov S, Bondar V, Puzyr A, Purtov K, Fitzgerald Z, Brenner DW (2009) Colloidal stability of modified nanodiamond particles. *Diam Relat Mater* 18:620–626. doi:10.1016/j.diamond.2008.10.049
- Grichko VP, Shenderova OA (2006) Nanodiamond: Designing the Bio-Platform. In: Shenderova OA, Gruen DM (eds) *Ultra-nanocrystalline diamond: synthesis, properties, and applications*. William Andrew Publ, Norwich, New York, pp 529–558
- Grichko V, Grishko V, Shenderova O (2006) Nanodiamond bullets and their biological targets. *NanoBiotechnology* 2:37–42. doi:10.1007/s12030-006-0005-8
- Gruber A, Dräbenstedt A, Tietz C, Fleury L, Wrachtrup J, von Borzyskowski C (1997) Scanning confocal optical microscopy and magnetic resonance on single defect centers. *Science* 276:2012–2014. doi:10.1126/science.276.5321.2012
- Guan B, Wu L, Ren B, Zhi J (2006) An easy method for attaching nanodiamond particles to amine active glass-like carbon. *Carbon* 44:2858–2860. doi:10.1016/j.carbon.2006.06.011
- Härtl A, Schmich E, Garrido JA, Hernando J, Catharino SCR, Walter S, Feulner P, Kromka A, Steinmüller D, Stutzmann M (2004) Protein-modified nanocrystalline diamond thin films for biosensor applications. *Nat Mater* 3:736–742. doi:10.1038/nmat1204
- Hens SC, Cunningham G, Tyler T, Moseenkov S, Kuznetsov V, Shenderova O (2008) Nanodiamond bioconjugate probes and their collection by electrophoresis. *Diam Relat Mater* 17:1858–1866. doi:10.1016/j.diamond.2008.03.020
- Hoch MJR, Reynhardt EC (1988) Nuclear spin-lattice relaxation of dilute spins in semiconducting diamond. *Phys Rev B* 37 (16):9222–9226. doi:10.1103/PhysRevB.37.9222
- Huang L-CL, Chang H-C (2004) Adsorption and immobilization of cytochrome *c* on nanodiamonds. *Langmuir* 20:5879–5884. doi:10.1021/la0495736
- Huang H, Pierstorff E, Osawa E, Ho D (2007) Active nanodiamond hydrogels for chemotherapeutic delivery. *Nano Lett* 7(11):3305–3314. doi:10.1021/nl071521o
- Huang H, Pierstorff E, Osawa E, Ho D (2008) Protein-mediated assembly of nanodiamond hydrogels into a biocompatible and biofunctional multilayer nanofilm. *ACS Nano* 2(2):203–212. doi:10.1021/nn7000867
- Hui YY, Cheng C-L, Chang H-C (2010) Nanodiamonds for optical bioimaging. *J Phys D: Appl Phys* 43:374021. doi:10.1088/0022-3727/43/37/374021
- Ida S, Tsubota T, Tanii S, Nagata M, Matsumoto Y (2003) Chemical modification of the diamond surface using benzoyl peroxide and dicarboxylic acids. *Langmuir* 19:9693–9698. doi:10.1021/la034133k
- Ikeda Y, Saito T, Kusakabe K, Morooka S, Maeda H, Taniguchi Y, Fujiwara Y (1998) Halogenation and butylation of diamond surfaces by reactions in organic solvents. *Diam Relat Mater* 7:830–834. doi:10.1016/S0925-9635(97)00304-X
- John P, Polwart N, Troupe CE, Wilson JIB (2003) The oxidation of diamond: the geometry and stretching frequency of carbonyl on the (100) surface. *J Am Chem Soc* 125:6600–6601. doi:10.1021/ja029586a
- Kalish R, Uzan-Saguy R, Philosph B, Richter V, Lagrange JP, Gheeraert E, Deneuille A, Collins AT (1997) Nitrogen doping of diamond by ion implantation. *Diam Relat Mater* 6:516–520. doi:10.1016/S0925-9635(96)00657-7
- Khabashesku VN, Margrave JL, Barrera EV (2005) Functionalized carbon nanotubes and nanodiamonds for engineering and biomedical applications. *Diam Relat Mater* 14:859–866. doi:10.1016/j.diamond.2004.11.006
- Knickerbocker T, Strother T, Schwartz MP, Russell JN Jr, Butler J, Smith LM, Hamers RJ (2003) DNA-modified diamond surfaces. *Langmuir* 19:1938–1942. doi:10.1021/bc0026279+
- Kong X, Huang LCL, Liao S-CV, Han C-C, Chang H-C (2005a) Polylysine-coated diamond nanocrystals for MALDI-TOF mass analysis of DNA oligonucleotides. *Anal Chem* 77:4273–4277. doi:10.1021/ac050213c
- Kong XL, Huang LCL, Hsu C-M, Chen W-H, Han C-C, Chang H-C (2005b) High-affinity capture of proteins by diamond nanoparticles for mass spectrometric analysis. *Anal Chem* 77:259–265. doi:10.1021/ac048971a
- Kossovsky N, Gelman A, Hnatyszyn HJ, Rajguru S, Garrell RL, Torbati S, Freitas SSF, Chow G-M (1995) Surface-modified diamond nanoparticles as antigen delivery vehicles. *Bioconjug Chem* 6(5):507–511. doi:10.1021/bc00035a001
- Kremers G-J, Gilbert SG, Cranfill PJ, Davidson MW, Piston DW (2011) Fluorescent proteins at a glance. *J Cell Sci* 124(2):157–160. doi:10.1242/jcs.072744
- Krueger A (2008) New carbon materials: biological applications of functionalized nanodiamond materials. *Chem Eur J* 14:1382–1390. doi:10.1002/chem.200700987
- Krueger A, Boedeker T (2008) Deagglomeration and functionalisation of detonation nanodiamond with long alkyl chains. *Diam Relat Mater* 17:1367–1370. doi:10.1038/nmat1204
- Krueger A, Ozawa M, Jarre G, Liang Y, Stegk J, Lu L (2007) Deagglomeration and functionalisation of detonation diamond. *Phys Status Solidi (A)* 204(9):2881–2887. doi:10.1002/pssa.200776330
- Krueger A, Stegk J, Liang Y, Lu L, Jarre G (2008) Biotinylated nanodiamond: simple and efficient functionalization of detonation diamond. *Langmuir* 24:4200–4204. doi:10.1021/la703482v

- Krüger A, Kataoka F, Ozawa M, Fujino T, Suzuki Y, Aleksenskii AE, Vul' AY, Ōsawa E (2005) Unusually tight aggregation in detonation nanodiamond: Identification and disintegration. *Carbon* 43(8):1722–1730. doi:10.1016/j.carbon.2005.02.020
- Krüger A, Liang Y, Jarre G, Stegk J (2006) Surface functionalisation of detonation diamond suitable for biological applications. *J Mater Chem* 16:2322–2328. doi:10.1039/b601325b
- Kurtsiefer C, Mayer S, Zarda P, Weinfurter H (2000) Stable solid-state source of single photons. *Phys Rev Lett* 85(2):290–293. doi:10.1103/PhysRevLett.85.290
- Lee SF, Osborne MA (2007) Photodynamics of a single quantum dot: fluorescence activation, enhancement, intermittency, and decay. *J Am Chem Soc* 129:8936–8937. doi:10.1021/ja071876+
- Li Y-q, Zhou X-p (2010) Transferrin-coupled fluorescence nanodiamonds as targeting intracellular transporters: An investigation of the uptake mechanism. *Diam Relat Mater* 19:1163–1167. doi:10.1016/j.diamond.2010.05.003
- Liang Y, Ozawa M, Krueger A (2009) A general procedure to functionalize agglomerating nanoparticles demonstrated on nanodiamond. *ACS Nano* 3(8):2288–2296. doi:10.1021/nm900339s
- Lidke DS, Arndt-Jovin DJ (2004) Imaging takes a quantum leap. *Physiology* 19:322–325. doi:10.1152/physiol.00030.2004
- Lim T-S, Fu C-C, Lee K-C, Lee H-Y, Chen K, Cheng W-F, Pai WW, Chang H-C, Fann W (2009) Fluorescence enhancement and lifetime modification of single nanodiamonds near a nanocrystalline silver surface. *PCCP: Phys Chem Chem Phys* 11:1508–1514. doi:10.1039/b817471g
- Liu YL, Sun KW (2010) Protein functionalized nanodiamond arrays. *Nanoscale Res Lett* 5:1045–1050. doi:10.1007/s11671-010-9600-7
- Liu Y, Gu Z, Margrave JL, Khabashesku VN (2004) Functionalization of nanoscale diamond powder: fluoro-, alkyl-, amino-, and amino acid-nanodiamond derivatives. *Chem Mater* 16:3924–3930. doi:10.1021/cm048875q
- Liu K-K, Cheng C-L, Chang C-C, Chao J-I (2007) Biocompatible and detectable carboxylated nanodiamond on human cell. *Nanotechnology* 18:325102. doi:10.1088/0957-4484/18/32/325102
- Liu K-K, Chen M-F, Chen P-Y, Lee T-J, Cheng C-L, Chang C-C, Ho Y-P, Chao J-I (2008) Alpha-bungarotoxin binding to target cell in a developing visual system by carboxylated nanodiamond. *Nanotechnology* 19:205102. doi:10.1088/0957-4484/19/20/205102
- Liu K-K, Wang C-C, Cheng C-L, Chao J-I (2009) Endocytic carboxylated nanodiamond for the labeling and tracking of cell division and differentiation in cancer and stem cells. *Biomaterials* 30(26):4249–4259. doi:10.1016/j.biomaterials.2009.04.056
- Liu K-K, Zheng W-W, Wang C-C, Chiu Y-C, Cheng C-L, Lo Y-S, Chen C, Chao J-I (2010) Covalent linkage of nanodiamond-paclitaxel for drug delivery and cancer therapy. *Nanotechnology* 21:315106. doi:10.1088/0957-4484/21/31/315106
- Lu M, Knickerbocker T, Cai W, Yang W, Hamers RJ, Smith LM (2004) Invasive cleavage reactions on DNA-modified diamond surfaces. *Biopolymers* 73(5):606–613. doi:10.1002/bip.20007
- Mangeney C, Qin Z, Dahoumane SA, Adenier A, Herbst F, Boudou J-P, Pinson J, Chehimi MM (2008) Electroless ultrasonic functionalization of diamond nanoparticles using aryl diazonium salts. *Diam Relat Mater* 17:1881–1887. doi:10.1016/j.diamond.2008.04.003
- Manus LM, Mastarone DJ, Waters EA, Zhang X-Q, Schultz-Sikma EA, MacRenaris KW, Ho D, Meade TJ (2010) Gd(III)-nanodiamond conjugates for MRI contrast enhancement. *Nano Lett* 10:484–489. doi:10.1021/nl903264h
- Maze JR, Stanwix PL, Hodges JS, Hong S, Taylor JM, Cappellaro P, Jiang L, Gurudev Dutt MV, Togan E, Zibrov AS, Yacoby A, Walsworth RL, Lukin MD (2008) Nanoscale magnetic sensing with an individual electronic spin in diamond. *Nature* 455:644–647. doi:10.1038/nature07279
- Miller JB, Brown DW (1996) Photochemical modification of diamond surfaces. *Langmuir* 12:5809–5817. doi:10.1021/la9407166
- Mitev D, Dimitrova R, Spassova M, Minchev C, Stavrev S (2007) Surface peculiarities of detonation nanodiamonds in dependence of fabrication and purification methods. *Diam Relat Mater* 16:776–780. doi:10.1016/j.diamond.2007.01.005
- Mitura S, Bakowicz K, Niedzielski P, Mitura E, Karczewska A, Sokolowska A, Szmidt J, Hassard J (2001) Inertness of diamond-truth or false. Paper presented at the 3rd International Conference on Novel Applications of Wide Bandgap Layers, 2002. IEEE Zakopane, Poland, pp 84–87
- Mkandawire M, Pohl A, Gubarevich T, Lapina V, Appelhans D, Rödel G, Pompe W, Schreiber J, Opitz J (2009) Selective targeting of green fluorescent nanodiamond conjugates to mitochondria in HeLa cells. *J Biophotonics* 2(10):596–606. doi:10.1002/jbjo.200910002
- Mochalin VN, Gogotsi Y (2009) Wet chemistry route to hydrophobic blue fluorescent nanodiamond. *J Am Chem Soc* 131:4594–4595. doi:10.1021/ja9004514
- Mohan N, Chen C-S, Hsieh H-H, Wu Y-C, Chang H-C (2010a) In vivo imaging and toxicity assessments of fluorescent nanodiamonds in *caenorhabditis elegans*. *Nano Lett* 10(9):3692–3699. doi:10.1021/nl1021909
- Mohan N, Tzeng Y-K, Yang L, Chen Y-Y, Hui YY, Fang C-Y, Chang H-C (2010b) Sub-20-nm fluorescent nanodiamonds as photostable biolabels and fluorescence resonance energy transfer donors. *Adv Mater* 22:843–847. doi:10.1002/adma.200901596
- Nakamura T, Ishihara M, Ohana T, Koga Y (2003) Chemical modification of diamond powder using photolysis of perfluoro-oazooctane. *Chem Commun* 900–901. doi:10.1039/B211807F
- Neugart F, Zappe A, Jelezko F, Tietz C, Boudou JP, Krueger A, Wrachtrup J (2007) Dynamics of diamond nanoparticles in solution and cells. *Nano Lett* 7(12):3588–3591. doi:10.1021/nl0716303
- Nguyen T-T-B, Chang H-C, Wu VW-K (2007) Adsorption and hydrolytic activity of lysozyme on diamond nanocrystallites. *Diam Relat Mater* 16:872–876. doi:10.1016/j.diamond.2007.01.030
- Nordsletten L, Högåsen AKM, Kontinen YT, Santavirta S, Aspenberg P, Aasen AO (1996) Human monocytes stimulation by particles of hydroxyapatite, silicon carbide and diamond: in vitro studies of new prosthesis coatings. *Biomaterials* 17:1521–1527. doi:10.1016/0142-9612(96)89777-8
- Osswald S, Yushin G, Mochalin V, Kucheyev SO, Gogotsi Y (2006) Control of sp²/sp³ carbon ratio and surface chemistry of nanodiamond powders by selective oxidation in air. *J Am Chem Soc* 128:11635–11642. doi:10.1021/ja063303n
- Petrov I, Shenderova O, Grishko V, Grichko V, Tyler T, Cunningham G, McGuire G (2007) Detonation nanodiamonds simultaneously purified and modified by gas treatment. *Diam Relat Mater* 16:2098–2103. doi:10.1016/j.diamond.2007.05.013
- Pramatarova L, Dimitrova R, Pecheva E, Spassov T, Dimitrova M (2007) Peculiarities of hydroxyapatite/nanodiamond composites as novel implants. *J Phys Conf Ser* 93:012049. doi:10.1088/1742-6596/93/1/012049
- Prawer S, Greentree AD (2008) Diamond for quantum computing. *Science* 320:1601–1602. doi:10.1126/science.1158340
- Purtov KV, Bondar VS, Puzyr AP (2001) Supramolecular structure of nanodiamond particles and obelin built up on a two-dimensional plate. *Dokl Biochem Biophys* 380:339–342. doi:10.1023/A:1012396327027
- Purtov KV, Petunin AI, Burov AE, Puzyr AP, Bondar VS (2010) Nanodiamonds as carriers for address delivery of biologically active substances. *Nanoscale Res Lett* 5:631–636. doi:10.1007/s11671-010-9526-0

- Puzyr AP, Baron AV, Purtov KV, Bortnikov EV, Skobelev NN, Mogilnaya OA, Bondar VS (2007) Nanodiamonds with novel properties: A biological study. *Diam Relat Mater* 16:2124–2128. doi:10.1016/j.diamond.2007.07.025
- Puzyr AP, Pozdnyakova IO, Bondar VS (2004) Design of a luminescent biochip with nanodiamonds and bacterial luciferase. *Phys Solid State* 46(4):761–763. doi:10.1134/1.1711469
- Puzyr AP, Purtov KV, Shenderova OA, Luo M, Brenner DW, Bondar VS (2007) The adsorption of aflatoxin B1 by detonation-synthesis nanodiamonds. *Dokl Biochem Biophys* 417:299–301. doi:10.1134/S1607672907060026
- Rabeau JR, Chin YL, Prawer S, Jelezko F, Gaebel T, Wrachtrup J (2005) Fabrication of single nickel-nitrogen defects in diamond by chemical vapor deposition. *Appl Phys Lett* 86:131926. doi:10.1063/1.1896088
- Resch-Genger U, Grabolle M, Cavaliere-Jaricot S, Nitschke R, Nann T (2008) Quantum dots versus organic dyes as fluorescent labels. *Nat Methods* 5(9):763–775. doi:10.1038/NMETH.1248
- Reynhardt EC, Terblanche CJ (1997) ^{13}C relaxation in natural diamond. *Chem Phys Lett* 269:464–468. doi:10.1016/S0009-2614(97)00309-6
- Rezek B, Shin D, Nakamura T, Nebel CE (2006) Geometric properties of covalently bonded DNA on single-crystalline diamond. *J Am Chem Soc* 128:3884–3885. doi:10.1021/ja058181y
- Saini G, Yang L, Lee ML, Dadson A, Vail MA, Linford MR (2008) Amino-modified diamond as a durable stationary phase for solid-phase extraction. *Anal Chem* 80:6253–6259. doi:10.1021/ac800209c
- Schrand AM, Huang H, Carlson C, Schlager JJ, Ōsawa E, Hussain SM, Dai L (2007) Are diamond nanoparticles cytotoxic? *J Phys Chem B* 111:2–7. doi:10.1021/jp066387v
- Schrand AM, Hens SAC, Shenderova OA (2009) Nanodiamond particles: properties and perspectives for bioapplications. *Crit Rev Solid State Mater Sci* 34:18–74. doi:10.1080/10408430902831987
- Shaner NC, Steinbach PA, Tsien RY (2005) A guide to choosing fluorescent proteins. *Nat Methods* 2(12):905–909. doi:10.1038/NMETH819
- Shenderova O, Petrov I, Walsh J, Grichko V, Grishko V, Tyler T, Cunningham G (2006) Modification of detonation nanodiamonds by heat treatment in air. *Diam Relat Mater* 15:1799–1803. doi:10.1016/j.diamond.2006.08.032
- Shimkunas RA, Robinson E, Lam R, Lu S, Xu X, Zhang X-Q, Huang H, Osawa E, Ho D (2009) Nanodiamond-insulin complexes as pH-dependent protein delivery vehicles. *Biomaterials* 30:5720–5728. doi:10.1016/j.biomaterials.2009.07.004
- Smith AH, Robinson EM, Zhang X-Q, Chow EK, Lin Y, Osawa E, Xi J, Ho D (2011) Triggered release of therapeutic antibodies from nanodiamond complexes. *Nanoscale* 3(7):2844–2848. doi:10.1039/c1nr10278h
- Spear KE, Dismukes JP (1994) *Synthetic diamond: emerging CVD science and technology*, vol 25. Wiley-IEEE Press, Piscataway
- Steinmüller-Nethl D, Kloss FR, Najam-Ul-Haq M, Rainer M, Larsson K, Linsmeier C, Köhler G, Fehrer C, Lepperdinger G, Liu X, Memmel N, Bertel E, Huck CW, Gassner R, Bonn G (2006) Strong binding of bioactive BMP-2 to nanocrystalline diamond by physisorption. *Biomaterials* 27:4547–4556. doi:10.1016/j.biomaterials.2006.04.036
- Strother T, Knickerbocker T, Russell JN Jr, Butler JE, Smith LM, Hamers RJ (2002) Photochemical functionalization of diamond films. *Langmuir* 18:968–971. doi:10.1021/la0112561
- Sushchev VG, Dolmatov VY, Marchukov VA, Veretennikova MV (2008) Fundamentals of chemical purification of detonation nanodiamond soot using nitric acid. *J Superhard Mater* 30(5):297–304. doi:10.3103/S1063457608050031
- Takahashi K, Tanga M, Takai O, Okamura H (2003) DNA preservation using diamond chips. *Diam Relat Mater* 12:572–576. doi:10.1016/S0925-9635(03)00070-0
- Thoms BD, Owens MS, Butler JE, Spiro C (1994) Production and characterization of smooth, hydrogen-terminated diamond C(100). *Appl Phys Lett* 65(23):2957–2959. doi:10.1063/1.112503
- Tomljenovic-Hanic S, Greentree AD, de Sterke CM, Prawer S (2009) Flexible design of ultrahigh-Q microcavities in diamond-based photonic crystal slabs. *Optics Express* 17(8):6465–6475. doi:10.1364/OE.17.006465
- Treussart F, Jacques V, Wu E, Gacoin T, Grangier P, Roch J-F (2006) Photoluminescence of single colour defects in 50 nm diamond nanocrystals. *Phys B* 376–377:926–929. doi:10.1016/j.physb.2005.12.232
- Tsubota T, Tanii S, Ida S, Nagata M, Matsuzawa M (2003) Chemical modification of diamond surface with various carboxylic acids by radical reaction in liquid phase. *Diam Relat Mater* 13:1093–1097. doi:10.1016/j.diamond.2003.10
- Ushizawa K, Sato Y, Mitsumori T, Machinami T, Ueda T, Ando T (2002) Covalent immobilization of DNA on diamond and its verification by diffuse reflectance infrared spectroscopy. *Chem Phys Lett* 351:105–108. doi:10.1016/S0009-2614(01)01362-8
- Vaijayanthimala V, Tzeng Y-K, Chang H-C, Li C-L (2009) The biocompatibility of fluorescent nanodiamonds and their mechanism of cellular uptake. *Nanotechnology* 20:425103. doi:10.1088/0957-4484/20/42/425103
- Wee T-L, Mau Y-W, Fang C-Y, Hsu H-L, Han C-C, Chang H-C (2009) Preparation and characterization of green fluorescent nanodiamonds for biological applications. *Diam Relat Mater* 18:567–573. doi:10.1016/j.diamond.2008.08.012
- Wei L, Zhang W, Lu H, Yang P (2010) Immobilization of enzyme on detonation nanodiamond for highly efficient proteolysis. *Talanta* 80:1298–1304. doi:10.1016/j.talanta.2009.09.029
- Weissleder R (1991) Target-specific superparamagnetic MR contrast agent. *Magn Reson Med* 22(2):209–212. doi:10.1002/mrm.1910220209
- Weissleder R (1999) Molecular imaging: exploring the next frontier. *Radiology* 212:609–614
- Weng M-F, Chiang S-Y, Wang N-S, Niu H (2009) Fluorescent nanodiamonds for specifically targeted bioimaging: Application to the interaction of transferrin with transferrin receptor. *Diam Relat Mater* 18:587–591. doi:10.1016/j.diamond.2008.07.012
- Williams OA, Hees J, Dieker C, Jäger W, Kirste L, Nebel CE (2010) Size-dependent reactivity of diamond nanoparticles. *ACS Nano* 4(8):4824–4830. doi:10.1021/nn100748k
- Wu E, Jacques V, Treussart F, Zeng H, Grangier P, Roch J-F (2006) Single-photon emission in the near infrared from diamond colour centre. *J Lumin* 119–120:19–23. doi:10.1016/j.jlumin.2005.12.052
- Xing Y, Dai L (2009) Nanodiamonds for nanomedicine. *Nanomedicine* 4(2):207–218. doi:10.2217/17435889.4.2.207
- Xu X, Yu Z, Zhu Y, Wang B (2005) Effect of sodium oleate adsorption on the colloidal stability and zeta potential of detonation synthesized diamond particles in aqueous solutions. *Diam Relat Mater* 14:206–212. doi:10.1016/j.diamond.2004.11.004
- Yang W, Auciello O, Butler JE, Cai W, Carlisle JA, Gerbi JE, Gruen DM, Knickerbocker T, Lasseter TL, Russell JN, Smith LM, Hamers RJ (2002) DNA-modified nanocrystalline diamond thin-films as stable, biologically active substrates. *Nat Mater* 1:253–257. doi:10.1038/nmat779
- Yeap WS, Tan YY, Loh KP (2008) Using detonation nanodiamond for the specific capture of glycoproteins. *Anal Chem* 80(12):4659–4665. doi:10.1021/ac800009v

- Yeap WS, Chen S, Loh KP (2009) Detonation nanodiamond: an organic platform for the suzuki coupling of organic molecules. *Langmuir* 25:185–191. doi:[10.1021/la8029787](https://doi.org/10.1021/la8029787)
- Yu S-J, Kang M-W, Chang H-C, Chen K-M, Yu Y-C (2005) Bright fluorescent nanodiamonds: no photobleaching and low cytotoxicity. *J Am Chem Soc* 127:17604–17605. doi:[10.1021/ja0567081](https://doi.org/10.1021/ja0567081)
- Yuan Y, Chen Y, Liu J-H, Wang H, Liu Y (2009) Biodistribution and fate of nanodiamonds *in vivo*. *Diam Relat Mater* 18:95–100. doi:[10.1016/j.diamond.2008.10.031](https://doi.org/10.1016/j.diamond.2008.10.031)
- Yuan Y, Wang X, Jia G, Liu J-H, Wang T, Gu Y, Yang S-T, Zhen S, Wang H, Liu Y (2010) Pulmonary toxicity and translocation of nanodiamonds in mice. *Diam Relat Mater* 19:291–299. doi:[10.1016/j.diamond.2009.11.022](https://doi.org/10.1016/j.diamond.2009.11.022)
- Zaitsev AM (2001) *Optical Properties of Diamond*. Springer, Berlin
- Zhang B, Li Y, Fang C-Y, Chang C-C, Chen C-S, Chen Y-Y, Chang H-C (2009a) Receptor-mediated cellular uptake of folate-conjugated fluorescent nanodiamonds: a combined ensemble and single-particle study. *Small* 5(23):2716–2721. doi:[10.1002/sml.200900725](https://doi.org/10.1002/sml.200900725)
- Zhang X-Q, Chen M, Lam R, Xu X, Osawa E, Ho D (2009b) Polymer-functionalized nanodiamond platforms as vehicles for gene delivery. *ACS Nano* 3(9):2609–2616. doi:[10.1021/nn900865g](https://doi.org/10.1021/nn900865g)
- Zhang X, Yin J, Kang C, Li J, Zhu Y, Li W, Huang Q, Zhu Z (2010) Biodistribution and toxicity of nanodiamonds in mice after intratracheal instillation. *Toxicol Lett* 198:237–243. doi:[10.1016/j.toxlet.2010.07.001](https://doi.org/10.1016/j.toxlet.2010.07.001)
- Zheng W-W, Hsieh Y-H, Chiu Y-C, Cai S-J, Cheng C-L, Chen C (2009) Organic functionalization of ultradispersed nanodiamond: synthesis and applications. *J Mater Chem* 19(44):8432–8441. doi:[10.1039/b904302k](https://doi.org/10.1039/b904302k)