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Nanodiamond as a drug delivery system: Applications and prospective

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ABSTRACT

With the rapid development of nanoscience and nanotechnology, a wide variety of nanomaterials have been synthesized and discovered. Revolutionary particles called nanodiamonds (NDs) have been considered for use in several medical applications due to its unique mechanical, chemical, optical, and biological properties. It has also sensing, imaging, and drug delivery properties. The study associated with the interface between ND and life sciences which is important for development of effective drug delivery systems.

Key words: Nanotechnology, nanomaterial, nanodiamond, drug delivery system.

INTRODUCTION

In recent years, a number of synthetic methods for the preparation of nanocrystalline diamond, “Nanodiamond” (ND), in the form of films and powders, have been developed (Dolmatov, 2001; Shenderova et al., 2002). Particularly, detonation synthesis, from powerful explosive mixtures (Greiner et al., 1988; Kuznetsov et al., 1994) has made such ND powder commercially available in ton quantities which has enabled many engineering applications and has expanded the application scope of diamond (Dolmatov, 2001). ND powders prepared by explosive techniques present a novel class of nanomaterials possessing unique surface properties. Due to the very small particle size (2-10 nm), a larger percentage of atoms in NDs contribute to the defect sites on grain boundaries than in single crystal natural or microcrystalline synthetic diamonds. For example, in individual 4.3 nm spherical particles of ND comprising about 7200 carbon atoms, nearly 1100 atoms are located at the surface (Shenderova et al., 2002). For this reason, the surface modifications of the nanosize diamond grains can affect the bulk properties of this material more strongly than those of micro- and macroscale diamonds. For example, ND powders can form good abrasive pastes and suspensions for high-precision polishing; ND-polymer composites are applied for manufacturing aircraft, cars and ships, as well as in hard and wear-resistant surface coatings. They are considered potential medical agents due to their high adsorption capacity, high specific surface area, and chemical inertness (Dolmatov, 2001; Shenderova et al., 2002). Applications of ND thin films have been demonstrated in the fabrication of cold cathodes, field emission displays (Choi et al., 1996; Ralchenko et al., 1999; Alimova et al., 1999; Jiang et al., 2002; Show et al., 2003), nanomechanical and nanoelectromechanical resonant structures (NEMS) (Wang et al., 2002; Sekaric et al., 2002; Philip et al., 2003), and were suggested for the design of biosensors as stable biologically active substrates after DNA-modification (Yang et al., 2002).

In order to minimize surface energy, individual ND particles (crystallites) of 4-6 nm size structurally self-organize into clusters or primary aggregates of 20-30 mm size. These, in turn, form larger weakly bonded secondary aggregates ranging from hundreds of nanometers to micron sizes. This agglomeration is likely facilitated by surface functional groups, such as —OH, —COOH, —SO_3H, and —NH_2, which are created along with other functionalities by chemical treatment processing of detonation-synthesized ND *(Jiang et al., 1996; Shenderova et al., 2002)* and participate in the formation of hydrogen bonds between ND clusters. However, for advanced applications of ND powder (e.g., in higher precision polishing compositions, nanoengineered electronic devices, polymer and ceramic composites, and bio-medical systems), the reduction of aggregate sizes to below 200 nm, and ultimately even to single clusters or particles, and the availability of specific functional groups on the surface is highly desirable. These functional groups can also serve as binding sites for covalent integration of ND into polymer structures and provide for improved solubility of ND powder in common solvents. Surface modification of the ND powder particles through a selective surface chemistry should be instrumental in approaching these goals. In view of the foregoing, functionalized ND powder will likely extend the utility of ND powder, and methods of making such functionalized ND powder will be in great demand.

Nanodiamond Structure

ND is an allotrope of carbon. NDs are carbon-based materials approximately 2 to 8 nanometers in diameter. Each ND's surface possesses functional groups that allow a wide spectrum of compounds to be attached to it, including chemotherapy agents. The crystal structure of ND consists of two close packed interpenetrating face centered cubic lattices; one lattice is shifted with respect to the other along the elemental cube space diagonal by one-quarter of its length (Figure 1) (Iakoubovskii et al., 2000; Iakoubovskii et al., 2008).

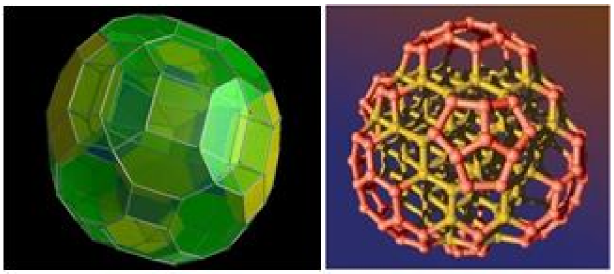

Fig 1: Crystalline structure of ND

The NDs also referred to as ultradisperse diamonds are particles in the 2-8 nm size range. ND is often described as a crystalline diamond core with a perfect diamond lattice surrounded by an amorphous shell with a combination of sp^2/sp^3 bonds or onion-like graphite shell **(**Shengfu et al., 1998). NDs are clustered carbon atoms with both graphitic (sp^2) and diamondoid (sp^3) bonds. The two types of bonds can be interchangeable, for example, the stretched face of diamond is a graphene plane. In reverse, the puckered graphene may become a diamond surface. This interchangeability allows ND particles to be flexible templates, particularly around the curved surface where electrons are unstable (Krueger, 2010).

NANODIAMONDS SYNTHESIS

In the phase diagram of carbon (Figure 2) there is a region at very high temperatures and pressure where diamond is stable. Hence, normally it is required to sustain according conditions for its production *(Krueger, 2010)*.

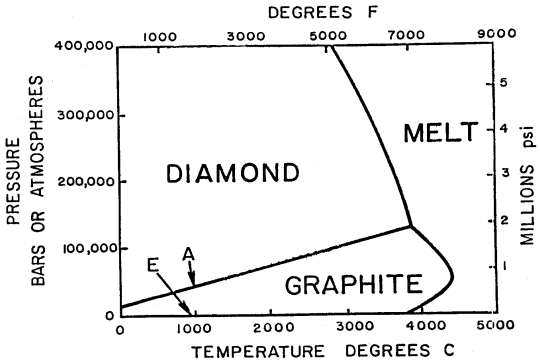

Fig 2: Carbon phase diagram.

NDs can be produced by different methods:

1. Detonation Nanodiamond

Detonation ND (DND), often also called ultradispersed diamond (UDD), is diamond that originates from a detonation. A ND can be created by detonating mixture of trinitrotoluene (TNT) and hexogen (RDX) (Figure 3) and then gathering the remaining soot.

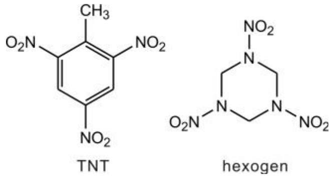

Fig 3: Structural formulae of the two explosives most commonly employed for detonation synthesis.

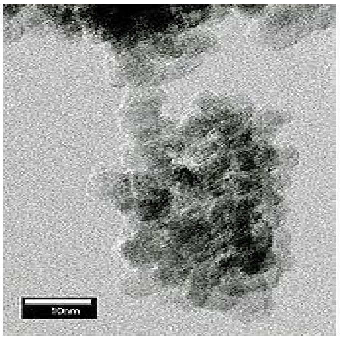

Fig 4: Detonation ND, TEM image

This idea is that pure ND can be produced by the detonation of a diamond blend and will then form by chemical purification. The soot left over actually contains tiny diamonds, which measure four nanometers in size. However, in order for these diamonds to shine and look anything like diamonds they must be exposed to a high-energy electron beam and then heated 800 degrees Celsius. The diamond yield after detonation crucially depends on the synthesis condition and especially on the heat capacity of the cooling medium in the detonation chamber (water, air, CO_2, etc.). The higher the cooling capacity, the larger the diamonds yield, which can reach 90%. After the synthesis, diamond is extracted from the soot using high-temperature high-pressure (autoclave) boiling in acid for a long period (ca. 1–2 days). The boiling removes most of the metal contamination, originating from the chamber materials, and non-diamond carbon. Various measurements, including X-ray diffraction *(Iakoubovskii* et al., *2000)* and high-resolution transmission electron microscopy *(Iakoubovskii* et al., *2008)* revealed that the size of the diamond grains in the soot is distributed around 5 nm. The grains are unstable with respect to aggregation and spontaneously form micrometer-sized clusters (Figures 4 and 5). The adhesion is strong and contacts between a few nano-grains can hold a micrometer-sized cluster attached to a substrate *(Iakoubovskii* et al., *2008)*.

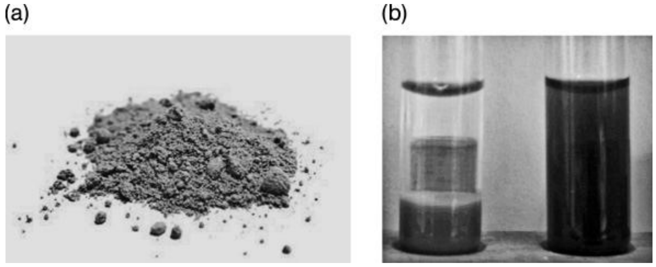

Fig 5: Detonation diamond as powder (a), as unstable suspension in water (center) and as completely deagglomerated dispersion in water (b).

2. Ultrasonic Cavitation Method

Diamond nanocrystals can also be synthesized from a suspension of graphite in organic liquid at atmospheric pressure and room temperature using ultrasonic cavitation. The yield is approximately 10%. The cost of NDs produced by this method is estimated to be competitive with the HPHT process *(Galimov et al., 2004; Khachatryan et al., 2008).*

3. Pulsed-laser irradiation:

An alternative synthesis technique is irradiation of graphite by high-energy laser pulses. The structure and particle size of the obtained diamond is rather similar to that obtained in explosion. In particular, many particles exhibit multiple twinning *(Shengliang et al., 2008).*

NANODIAMONDS PROPERTIES

ND has many unique physical characteristics just like its bigger cousin, the full karat diamond stone. The following superior properties of ND make it a special and promising material that can be widely applied in numerous fields:

1. Hardness

Table 1 shows some physical properties of diamond compared to Titanium and stainless steel. The hardness of diamond is about 50 times of Titanium and stainless steel. The toughness of diamond makes it suitable in applications in biomedical fields such as implant, cutting tools for surgeries, etc.

Table 1: Comparison of properties of chemical vapor deposited (CVD) diamond, titanium and stainless steel (316).

Properties	**CVD diamond**	**Titanium**	**Stainless steel**
Hardness (kg/mm2	10,000	230	210
Young's modulus (GPa)	1000	120.2	215.3
Bulk modulus (GPa)	442	108.6	166
Thermal conductivity (Watts/cm.°C)	20	0.21	0.16 3.8 (***Cu***)
Thermal expansion ($\times 10^{-8}$ K^{-1})	1.1	8.8	17.2
Coefficient of friction	0.05	0.1 (***Graphite***)	
Refractive index (in the near IR)	2.41- 2.44	3.42-3.5 (***Si***)	

Source: From Ref. (Tang et al., 1995).

2. Chemical inertness is an important factor for ND to be applied in biology, since the biological environment is corrosive. Alloy of Ti_6Al_4V coated with ND films show that the diamond films have a very good chemical resistance to the corrosive liquid *(Azevedo et al., 2005).*

3. Biocompatibility cannot be ignored when diamond is applied to biology. Yu and coworkers investigated the biocompatibility of fluorescent ND (FND) powder with size of 100nm in cell culture and found low cytotoxicity in kidney cells *(Yu et al., 2005).* Further, Schrand and coworkers showed that ND with small size of 2-10nm are not toxic to a variety of cells through mitochondrial function (MIT) and luminescent ATP production assays *(Schrand et al., 2007).* It was found that after the incubation of cells with NDs, cell morphology is unaffected by the presence of NDs while NDs are seen surrounding the cell borders and attached to neurite extensions.

4. Excellent optical property is necessary for diamond to be applied as a biomarker or a biolabel. There are impurity sites within core, defects in the diamond or sp^2 clusters on the ND surface. With the light excitation, the ND will emit light with different frequency due to different type of impurity sites *(Holt, 2007).*

5. Chemical modification of diamond surface is essential for diamond to be applied as potential biosensor or biochip *(Guan et al., 2006)*, or a substrate to immobilize biological molecules. Diamond surface can be hydrogen-terminated by exposing the surface to 13.5-MHz inductively coupled hydrogen plasma (15 torr) at 800°C *(Thoms et al., 1994).* With the hydrogen-terminated nanocrystalline diamond, Yang and coworkers successfully designed a chemical procedure to attach DNA onto the diamond surface. Recently, ND with the size of 5-100nm in diameter was carboxylated by *Chang and Han, (2006).* It was found that carboxylated ND *(Holt, 2007)* has good physical absorption properties including hydrophobic and hydrophilic interaction, which can be used to immobilize biomolecules.

6. Tiny size and ability to control the constancy of the nano-size and the shape of agglutinates.

7. Large Surface area and high adsorption potential:

It has extremely large relative surface area. As a result, its surface spontaneously attaches water and hydrocarbon molecules from the ambient atmosphere *(Shengfu et al., 1998)* and also larger number of drugs can be placed on the particles. However, clean ND surface can be obtained with appropriate handling *(Iakoubovskii* et al., *2008)*.

8. Outstanding photoluminescence.

NANODIAMOND APPLICATIONS AND PROSPECTIVE

Owing to its properties, ND like the classical diamond is an attractive material for many applications. They include the preparation of composites and coatings, mechanical applications to reduce friction or to modify surfaces, uses in electro-deposition or biomedical applications.

1. Mechanical Applications

- Super smooth polishing of gems, ceramics, glass, silicon wafers, and surgical knives *(Sung and Lin, 2010)*. So, it is widely used in various polishing compositions (pastes, gels and slurries) for obtaining especially smooth surfaces in production of computer hard discs; lenses, prisms, mirrors, etc. for various optical devices; high precision parts made of steel and hard alloys; silicon wafers; and/or body implants.
- Reinforcement of rubber, resins, plastic, PTFE, and metals (Cu, Al) *(Shenderova et al., 2002)*. NDs are used as active fillers in polymers providing improvement of various service characteristics as strength, elasticity, and increasing heat conductivity; and improving the optical characteristics of polymers.
- Lubrication of engine oil and machine grease: ND can reduce significantly the frictional coefficient by coating the sliding surface.
- Electroplating of metal coating to provide markedly improved mechanical properties of the metal *(Sung and Lin, 2010)* in production of punches, dies, matrices and molds; screws, check valves, pistons and spruces; metal cutting tools; and /or food processing equipment.

2. Thermal Applications

The high thermal conductivity can be employed for ND applications as well. ND is a suitable additive for coolants to improve the thermal conductivity of cooling media. An addition of only 0.3% to cooling oils for large transistors causes a 20% growth of thermal conductivity. This effect serves to prevent the formation of hot zones inside the coolant and the consequent destruction of the transistor *(Krueger, 2010)*. So, ND efficiently enhances material's ability to dissipate heat. Consequently, using ND in pastes, glues and substrates provides an exceptional opportunity of avoiding burnout, increasing speed of active elements, reducing the size of devices, and increasing their reliability and durability.

3. Electrochemical Applications

Due to its physicochemical stability, large electrochemical potential window, and chemical sensitivity *(Prado et al., 2002)*, diamond is an excellent candidate for electrochemical applications. Diamond electrodes show the most stable response among electrodes by far, and do not require extensive pretreatment to regenerate the electroactive surface *(Halpern et al., 2006)*. ND electrodes/microelectrodes have been applied to biological system as biosensors *(Martinez-Huitle, 2007)*.

4. Cosmetic Applications

As ND is non-poisonous, a big area of application is ND-impregnated cosmetics. So, ND can be formulated as a dental filling, lotion, deodorant, toothpaste, shampoo, antibiotic, dermal strip, skin cleanser, or exfoliant.

4.1. Dental care

ND may be formulated as a dental material. The dental material can be formulated for use as a filling, veneer, reconstruction, and the like. The ND particles can provide additional mechanical strength, as well as an appearance that approximates natural enamel when dry. Alternatively, the remedial healthcare composition can be formulated as toothpaste including an acceptable carrier and a plurality of ND particles. ND added toothpaste has another advantage, as ND is known to cure gum disease *(Sung and Lin, 2010)*.

4.2. Skin Care

Owing to unique adsorption capabilities of ND, their addition to skincare products enhances the effect of biologically active ingredients and facilitates their penetration into deeper skin layers. ND makes all biologically-active additives "work" at their maximum efficiency. Also, due to their unique optical properties, ND is an excellent agent for skin protecting from harmful UV radiation.

The ND surface functional groups form powerful bonding with water molecules to provide an all-day-long moisturizing effect and to protect the skin from aging. At the same time, ND-base creams are fully and rapidly absorbed by the skin.

ND compositions can be formulated for skin care products such as lotions, facial tissue lotion, deodorant, dermal strip, skin cleanser, soap, and exfoliant. The ND particles can be dispersed in a biologically acceptable carrier and contacted with a biological material such as organic oils, sebum, bacteria, epithelial cells, amino acids, proteins, DNA, and combinations thereof. Once the biological material is bonded to ND, the ND composition can be removed from the surface or environment. The ND composition can then be discarded or further treated to identify or otherwise utilize the absorbed biological material.

The presence of ND particles can improve absorption of oils and undesirable deposits from the skin without abrasiveness associated with larger diamond particles. The NDs can be present in the facial tissue lotion, deodorant, dermal strip, and skin cleanser. In addition, the skin cleanser can include additives such as fragrance, colorants, vitamin E, herbal supplements, antibiotics, UV absorbers, hydrating agents, sun-block agents, exfoliating agents, and the like. Also, NDs in antibiotic and lotion compositions can increase healing of skin and removal of damaged skin such as with sunburns and scar tissue *(Sung and Lin, 2010)*.

4.3. Hair and nail care

Shampoo can include an acceptable carrier and ND particles. Suitable bubbling agents can be included to increase contact of unsaturated NDs with a biological material. This can be advantageous in maximizing the effect of NDs in skin cleansers, deodorants, shampoos, soaps, toothpaste, etc...

Another cosmetic ND composition can be formulated as a nail polish, eyeliner, lip-gloss, or exfoliant. ND particles can also improve the durability of the applied nail compositions. Specifically, NDs can provide increased resistance to chipping and wear, e.g. typically a ND nail polish can last from about three to ten time longer than typical nail lacquer formulations *(Sung and Lin, 2010)*.

5. Biological Applications

ND is the state-of-the-art material rapidly finding its way into biotechnologies such as:

5.1. Nanodiamond in biomedical applications

Due to its hardness, chemical inertness, thermal conductivity, and low cytotoxicity, ND could be applied as coating materials of implants, other surgery tools, etc. in biomedical fields. In 1995, for the first time, Zolyfinski and coworkers implanted orthopedic screws, coated with nanocrystalline diamond film (NCD) to a patient with a complex fracture of femoral bone *(Zolyfinski et al., 1996)*. After surgery, no ejection was observed, whereas the standard metal implants were rejected twice. As such, diamond is ideal for use in medical applications, e.g. artificial replacements (joint coatings, heart valves, *etc.*), and will not deteriorate over time. Another implantation application of diamond is that an endoprothesis of hip joint, coated with NCD film was successfully implanted to a living organism *(Mitura et al., 2006)*.

5.2. Immobilization of biomolecules

The chemical modification and physical absorption of diamond surface hold promises for ND to be applied in immobilization of protein and DNA for purification, separation, and further analysis (Figure 6). Using detonation ND, Bondar and coworkers *(Bondar et al., 2004)* successfully separated recombinant Ca^{2+}-activated photoprotein apoobelin and recombinant luciferase from bacterial cells of *Escherichia coli* through physical absorption of proteins on ND. For traditional purification by chromatographic means, it usually takes several days. The procedures using ND, the whole process took 30-40 min with a yield of 35-60%. Kong and coworker applied the same principle to capture proteins and DNA for the matrix-assisted laser desorption/ ionization (MALDI) time of fight (TOF) mass spectrometry (MS).

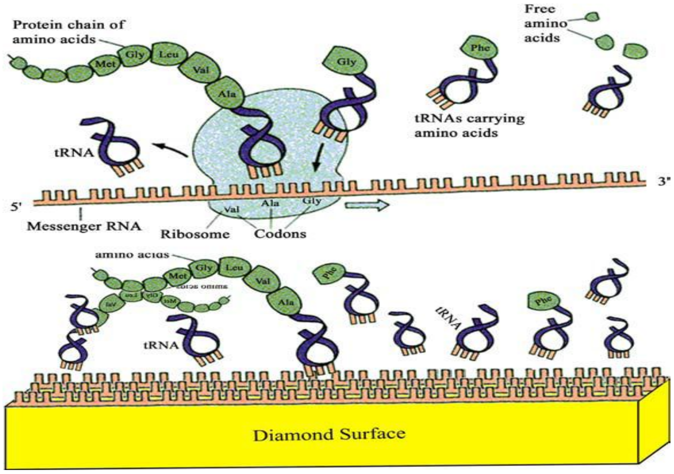

Fig 6: The effective protein manufacture by biological ribosome (top) and the chancy protein formation on diamond surface (bottom).

Carboxylated ND exhibits high affinity to proteins and polypeptides through hydrophobic and hydrophilic forces. Proteins in very dilute solution can be easily captured by ND and separated and directly analyzed by MALDI-TOF-MS *(Kong et al., 2005a; Kong et al., 2005b)*.

Puzyr and coworkers designed a luminescent biochip with NDs and bacterial luciferase. It is demonstrated that the enzyme in this structure retains the catalytic activity from recording the luminescent signal. The luminescent intensity is sufficient high for this biochip to be used in bioluminescent analysis *(Puzyr et al., 2004)*.

5.3. Applications as excellent Sorbent

Materials like activated carbon have been known for long to possess good adsorptive properties, especially when they exhibit a large active surface. The specific surface of ND ranges up to 300 $m^2\ g^{-1}$, which should render it attractive for this type of application, too. It may adsorb up to four times its own weight of water, so it is a suitable additive in certain areas of application. Yet it is not only water, but also other substances (like with biological origin) that can be adsorbed to the ND surface. Each carbon atom on the surface of ND has at least one dangling electron that may bond to a light element, such as H, N, or O. As biological materials are made of carbon compounds, almost all life sustaining chemicals can be absorbed by ND. Thus, ND is an excellent absorbent for amino acids, proteins, platelets, and DNA (Figure 7).

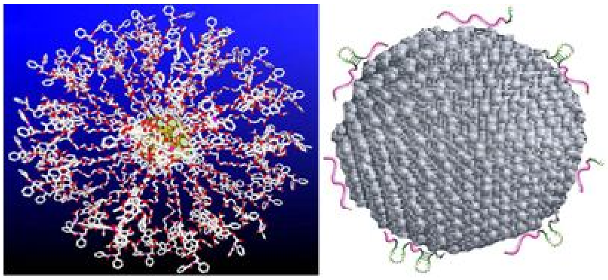

Fig 7: The diamondoid carrier with DNA strands anchoring on its surface (left); The flexible fitting of RNA on diamond surface might allow the first assembling of viruses by shrouding it with proteinoids (right).

Although diamond is highly stable, if the ND surface is free of adsorbent or absorbent, i.e. clean, it is thought that carbon atoms on the surface contain unpaired electrons that are highly reactive. As a result, ND particles can readily bond to and effectively absorb a variety of atomic species. For example, small atoms such as H, B, C, N, O, and F can be readily adsorbed on the ND surface, although other atoms can also be absorbed. In addition, those small atoms are building blocks, e.g. H, CO, OH, COOH, N, CN and NO, of organic materials including biological molecules. Consequently, ND particles can readily attach to amino acids, proteins, cells, DNA, RNA, and other biological materials, and ND particles can be used to remove skin oils, facial oils, compounds that result in body odor, bacteria, etc.

Further, NDs are typically smaller than most viruses (10–100 nm) and bacteria (10–100μm). Therefore, ND can be used to penetrate the outer layers of viruses and bacteria and then attach to RNA, DNA or other groups within the organism to prevent the virus or bacteria from functioning. Human body contains about 1/4 of carbon by weight. ND as carbon is non-poisonous. Moreover, it is not only cancer inactive, but also a catalyst for promoting drug effectiveness. For examples, ND has been used to treat burning skin infections, food poisons, and intestine malfunctions with good results. In comparison to tiny ND particles, the human cells appear to be colossal (Figure 8). Much larger than microbes, ND cannot harm normal cells. ND cannot penetrate the cell’s membrane. On the contrary, ND can stick to the DNA of bacteria or RNA of viruses. It is believable that ND may also be effective in attaching genes and hence it is capable to kill drug resistant viruses (e.g. HIV, SARS).

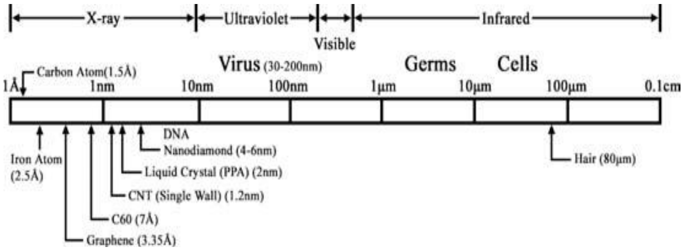

Fig 8: The relative scale of ND compared to microbes and cells.

ND surfaces may be modified by the termination of various chemical radicals (Figure 9). If the termination is nonpolar (e.g. H or F), the surface is hydrophobic. On the other hand, if hydrogen bonds may be formed on the absorbents (e.g. O or S), the surface is hydrophilic. The water repelling or wetting behavior is important for dispersion in liquid. The surface modification also allows the attachment of organic molecules, such as amine, carboxyl, carbonyl, hydroxyl, amide, nitrile, sulfide, epoxyl, phosphryl, sulfate, imide, etc.

For ND just formed, the surface contain ample C–O, C=O, C–N, C=N, and OH. Although these radicals are wettable by water, they tend to agglomerate. After boiling in sulfuric acid, the dispersion in water improved, so is the dispersion. However, if an organic polymer is used to as the matrix material, ND should be heat treated beforehand in hydrogen, fluorine or chlorine to render the surface hydrophobic *(Sung and Lin, 2010)*.

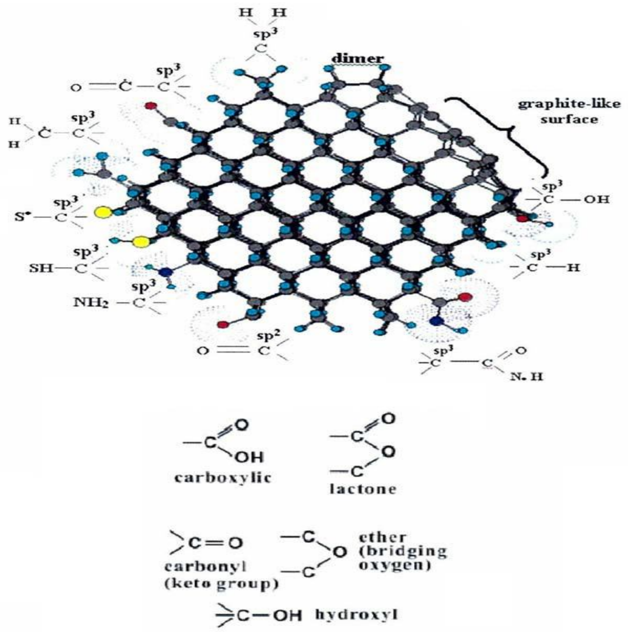

Fig 9: The attachment of surface absorbents on ND (top diagram) and the major surfactant radicals (bottom diagram).

5.4. Biomarkers and Biolabeling

ND has a very strong fluorescence at 700nm is emitted after excited at 560nm *(Yu et al., 2005)* due to the nitrogen-vacancy (N-V) center within diamonds. This is advantageous for imaging in biological cells, as the background fluorescence in cell is 300-400 nm *(Holt, 2007)*. It was found that under the same excitation conditions, the fluorescence of a single 35nm diamond is significantly brighter than that of a single dye molecule such as Alexa Fluor 546. Fluorescent ND (FND) coated with poly-L-lysine (PL) was used to study the interaction between DNA and FND on an amine-terminated glass substrate *(Fu et al., 2007)*. The PL was used to facilitate the binding of DNA (fluorescently labeled with TOTO-1 dye molecule) to FND. Due to the specific strong fluorescence at 700nm of FND, the DNA molecule is wrapped around the PL-coated FND particle. Fu and coworkers also demonstrated that it is possible to conduct a single particle tracking for a 35-nm FND in the cytoplasm of a live Hela cell. This tracking method could be applied to drug delivery system of ND, where the interaction between ND and cell could be monitored using fluorescence microscopy. Raman spectrum of diamond exhibits a sharp peak at 1332 cm^{-1} and the peak is isolated and the Raman absorption cross section is large *(Knight and White, 1989)*. This peak can be used as an indicator of the location of ND. Also, Cheng and co-workers tracked growth hormone factor in one single cancer cell using ND growth hormone complex (cND-rEaGH) as a specific probe *(Cheng et al., 2007)*.

5.5. Nanodiamond could be used in disease diagnosis

Like ordinary diamonds, NDs have tiny holes, known as vacancies, in which a nitrogen atom has replaced a carbon. High nitrogen content gives natural diamonds a yellowish tint, and the NDs absorb yellow light and emit violet (Figure 10).

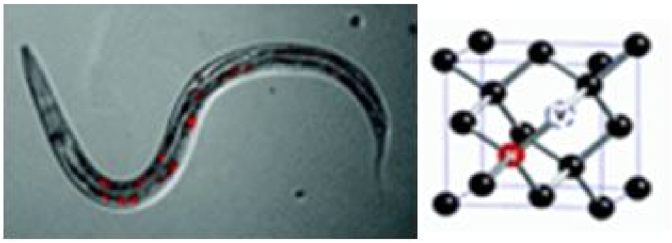

Fig 10: Fluorescent nanodiamond in diagnosis of disease.

NDs coated with sugars or proteins (such as dextran and bovine serum albumin), and then introduced both coated and uncoated types to transparent round worms (*Caenorhabditis elegans*). The NDs were either fed to the worms as a colloidal suspension or micro-injected into the gonads. It was found that the plain NDs fed to the worms remained within the digestive tract, coating its internal surfaces, while the coated NDs were able to pass through the tract walls and into the worm's body, collecting at several points. The location of the NDs was shown up as violet light when yellow light was shone on the worms. When injected into the gonads, the NDs were dispersed in the gonad and passed on to the embryos and eventually to the hatched larvae. In addition, all the worms in the study went on to live normal life spans with normal reproduction, and none showed any sign of distress.

For this reason, the researchers showed NDs were safe, stable, and non-toxic at cellular and organ levels. The NDs in the study were coated with sugars or proteins, but the scientists said almost any form of chemical could be used to coat them. The coated NDs could then migrate to target cells such as pathogens, immune system cells or cancer cells. This process could initially be used to map out cancers or other disease conditions, but could eventually be used to deliver low doses of drugs directly to the target tissues. Moreover NDs could be applied for imaging and tracking human stem cells that might even be able to regenerate entire organs if the research is successful *(Mohan et al., 2010)*.

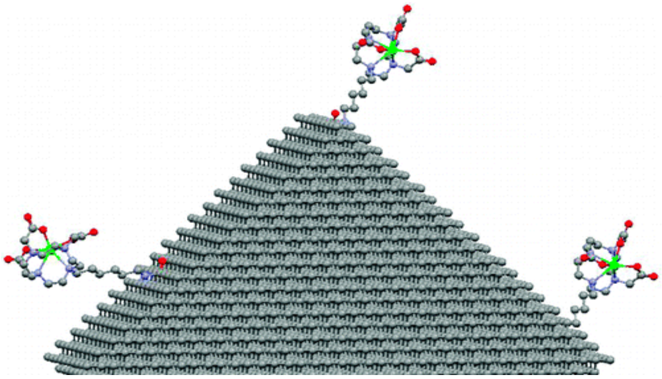

Fig 11: A Gd(III)-ND conjugate [Gd(III)-ND] was prepared and characterized, enabling detection of NDs by MR imaging.

5.6. Nanodiamond improves image resolution of MRI

Magnetic resonance imaging (MRI) is a noninvasive medical imaging technique that uses an intravenous contrast agent to produce detailed images of internal structures in the body. Contrast agents are used in MRI because they improve image resolution. A Northwestern University study shows that coupling a MRI contrast agent to a ND results in dramatically enhanced signal intensity and thus bright image contrast. Gadolinium (Gd) is the material most commonly used as an MRI contrast agent, but its contrast efficacy can be improved by conjugation with ND. Gd (III)-ND complex (Figure 11) demonstrated a greater than 10-fold increase in improvement of image resolution and, in turn, a significant increase in contrast enhancement *(Manus et al., 2010)*.

5.7. Drug Delivery Vehicles

Recently, the uptake of ND by living cell found in the biological ND research facilitated the use of NDs as drug carriers and delivery vehicles. NDs possess numerous hallmarks of an ideal drug delivery system and are promising platforms for advancing cancer therapy. They're nontoxic, and the body's immune system doesn't attack them. They can bind tightly to a variety of molecules and deliver them right into a tumor. In 1995, Kossovsky and coworkers used ND coated with cellobiose, a disaccharide, to immobilize mussel adhesive protein (MAP) antigen *(Kossovsky et al., 1995)*. Then the diamond-cellobiose-MAP complex was injected into NewZealand white rabbits which have their specificity against MAP. The delivery of antigen caused a strong and specific antibody response. Further experiments showed that ND immobilization resulted in maintaining the protein conformations allowing better antibody binding and hence a strong immune response. Moreover, the drug-ND complexes had no negative effect on the white blood cell count. This is especially important for cancer treatment: if the white blood cell count drops below a certain level, treatment is stopped due to the risk of major complications *(Liu et al., 2007)*.

For the first time, Huang and coworkers demonstrated that NDs serve as efficient chemotherapeutic drug carriers. ND materials can serve as highly versatile platforms for the controlled functionalization and delivery of a wide spectrum of therapeutic elements. In this work, doxorubicin hydrochloride (DOX), an apoptosis-inducing drug widely used in chemotherapy, was successfully applied toward the functionalization of ND and introduced toward murine macrophages as well as human colorectal carcinoma cells with preserved efficacy. The adsorption of DOX onto the NDs and its reversible release were achieved by regulating Cl^- ion concentration (Figure 12), and the NDs were found to be able to efficiently transport the drug inside living cells *(Huang et al., 2007)*.

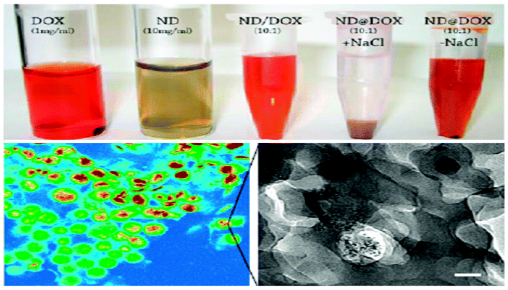

Fig 12: Nanodiamond hydrogels as efficient chemotherapeutic delivery vehicles.

Measurement of pH dependent cancer therapeutic interactions with ND carrier

In this work, we have combined constant-pH molecular dynamics simulations and experiments to provide a quantitative analysis of pH dependent interactions between doxorubicin hydrochloride (DOX) and faceted ND carriers.

This study suggests that when a mixture of faceted ND and DOX is dissolved in a solvent, the pH of this solvent plays a controlling role in the adsorption of DOX molecules on the ND. We find that the binding of DOX molecules on ND occurs only at high pH (Figure 13) and requires at least ~10% of ND surface area to be fully titrated for binding to occur. As such, this study reveals important mechanistic insight underlying an ND-based pH-controlled therapeutic platform *(Adnan et al., 2011).*

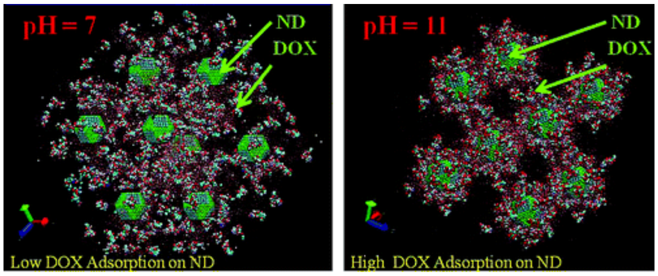

Fig 13: Effect of pH on the adsorption of DOX on ND.

Nanodiamonds enhance the effectiveness of the anti-tumor medicine

Chemotherapy, the therapeutic introduction of chemical toxins to cancerous tissue, can lose its effectiveness relatively quickly. Due primarily to a strongly conserved, pumping mechanism, known as *cell efflux*, chemo-resistant tumor cells are able to rapidly pump the anti-tumor chemicals out of the cell, rendering the chemotherapy much less effective. Pumped-out chemotoxins building up in extracellular tissue and sometimes killing healthy cells. This adds both to the pain, and cost of chemotherapy. The key to enhancing the therapy lies in the chemistry and the geometry of simple carbon crystals known as NDs. A team of bio-medical engineers led by Dean Ho of Northwestern University (Evanston, Illinois), are using NDs to cleverly use a flaw in the cell’s pumping system and boost the effectiveness of the anti-tumor medicine.

The diamonds are all eight-sided carbon structures “truncated octahedrals” measuring just a few billionths of a meter in size (hence *nano*). These nanostructures likewise have facets, with some facets carrying a negative charge and others being electrically neutral. This means that an additional chemical, such as the chemotherapeutic agent doxorubicin, can be attached to them, and then later, inside the cell, be released and dispersed. NDs have another useful property: their geometry doesn’t fit with the shape of the proteins that make up the tumor cell’s efflux pump. Hence, the diamonds stay inside the cell longer, boosting the effectiveness of the chemotherapy treatment, and inducing greater cell death. What’s more, the use of NDs as agents for delivery seems to greatly reduce the toxic side effects of the therapy.

The team administered two different treatments to two groups of mice with liver tumors, the first treatment consisted of the ND-doxorubicin combo (called NDX), and the second with doxorubicin alone. After two days the liver tumors were biopsied and analyzed. The results: mice treated with the ND compound had cellular levels of doxorubicin ten times higher than those mice treatment with the chemo drug alone. Further, tumor size was reduced more and life span extended in mice receiving NDX. An additional experiment was performed using the NDX preparation on mammary carcinoma models with similar positive results. Thus, ND-conjugated chemotherapy represents a promising, biocompatible strategy for overcoming chemoresistance and enhancing chemotherapy efficacy and safety *(Chow et al., 2011).*

Nanodiamonds as intracellular transporters of chemotherapeutic drug

To explore the application of NDs as anticancer delivery vehicles, firstly we examined the toxicity of NDs to three kinds of mammalian cells. The studies indicated that serum proteins in cell culture medium had significant effect (Figure 14).

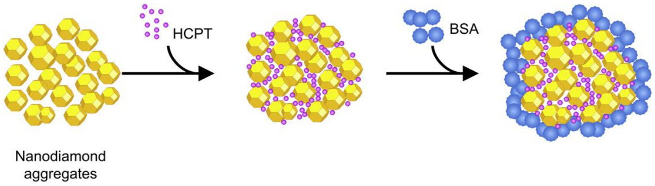

Fig 14: Schematic illustration showing the different loading of HCPT and BSA on NDs.

When cells were exposed to NDs dispersed in complete cell culture medium, no cytotoxicity was detected. However, severe cell death was found after exposed to NDs dispersed in serum-free medium. Possible reasons for serum-dependent cytotoxicity were discussed and the potential influence of serum on the test of efficacy was pointed for anticancer delivery system based on NDs. Then adsorption of anticancer 10-hydroxycamptothecin (HCPT) by NDs was studied. Experiments indicated that diluted NaOH solution could promote the loading of HCPT on NDs and a slow release of HCPT from the HCPT-ND complex was established in low pH media. Assessments of cell viability and imaging with transmission electron microscopy demonstrated a much higher efficacy of the HCPT-ND complex compared with HCPT alone. On the basis of adsorption feature of ND aggregates, new strategy for the design of targeting drug delivery systems of NDs was proposed *(Li et al., 2010).*

Nanodiamond-insulin administration

One medical use for the nanoparticles is to administer insulin, which acts as a growth hormone, into the body to help fight infection after wounded. The NDs with insulin can then be put in gels, ointments, and bandages. Since NDs tend to cluster naturally after extraction, thus having a relatively large surface area, large amounts of insulin can be placed on the NDs. When ND-insulin clusters are in an environment with a slightly basic pH, the insulin releases itself from the NDs. Because a bacterially infected wound has a pH higher than the physiological pH of 7.4, the insulin will only release where the infected area is. Since localized release of therapeutic medicine is becoming more and more important *(Wu et al., 2008).*

Safe gene therapy with NDs

Gene therapy holds promise in the treatment of a myriad of diseases, including cancer, heart disease and diabetes, among many others. However, developing a scalable system for delivering genes to cells both efficiently and safely has been challenging. The power of NDs as a novel gene delivery technology that combines key properties in one approach: enhanced delivery efficiency along with outstanding biocompatibility. The application of NDs for chemotherapeutic delivery and subsequently discovered that the NDs also are extremely effective at delivering therapeutic proteins *(Cui et al., 2005).* A research team engineered surface-modified ND particles that successfully and efficiently delivered DNA into mammalian cells (Figure 15).

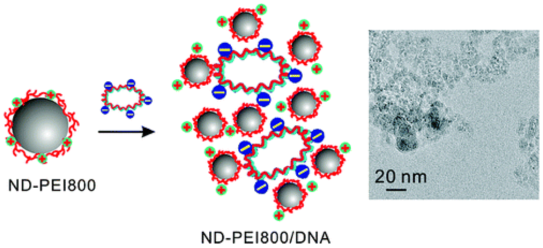

Fig 15: Surface-modified ND with PEI800 delivered DNA into cells.

The delivery efficiency was 70 times greater than that of a conventional standard for gene delivery. The new hybrid material could impact many facets of nanomedicine *(Zhang et al., 2009).* Functional NDs are rapidly emerging as promising carriers for next-generation therapeutics with demonstrated potential. NDs introduced as vectors for *in vitro* gene delivery *via* surface-immobilization with 800 Da polyethyleneimine (PEI800) and covalent conjugation with amine groups. PEI800-modified NDs exhibited the high efficiency of high molecular weight PEI (PEI25K), but without the high cytotoxicity inherent to PEI25K. Additionally, it was demonstrated that the enhanced delivery properties were exclusively mediated by the hybrid ND−PEI800 material and not exhibited by any of the materials alone. This platform approach represents an efficient avenue toward gene delivery *via* DNA-functionalized NDs, and serves as a rapid, scalable, and broadly applicable gene therapy strategy.

Nanodiamond-mediated delivery of water-insoluble therapeutics

A broad array of water-insoluble compounds has displayed therapeutically relevant properties toward a spectrum of medical and physiological disorders, including cancer and inflammation. However, the continued search for scalable, facile, and biocompatible routes toward mediating the dispersal of these compounds in water has limited their widespread application in medicine. This study demonstrated a platform approach of water-dispersible nanodiamond cluster-mediated interactions with several therapeutics to enhance their dispersion in water and preserve their functionality (Figure 16). These therapeutics include Purvalanol A, a highly promising compound for hepatocarcinoma (liver cancer) treatment, 4-hydroxytamoxifen (4-OHT), an emerging drug for the treatment of breast cancer, as well as dexamethasone, a clinically relevant anti-inflammatory that has addressed an entire spectrum of diseases that span complications from blood and brain cancers to rheumatic and renal disorders. Given the scalability of nanodiamond processing and functionalization, this novel approach serves as a facile, broadly impacting and significant route to translate water-insoluble compounds toward treatment-relevant scenarios *(Chen et al., 2009).*

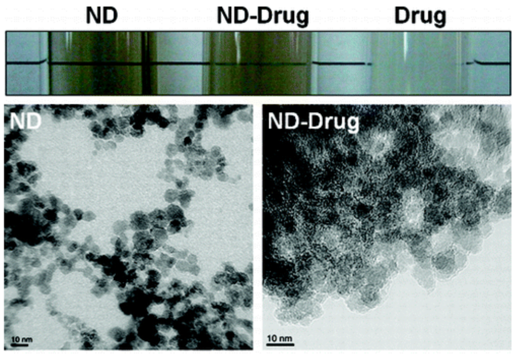

Fig 16: ND cluster-mediated interaction with water insoluble drugs.

Nanodiamonds between biocompatibility and cytotoxicity

Because of their unique photoluminescence and magnetic properties, NDs are promising for biomedical imaging and therapeutic applications. However, these biomedical applications will hardly be realized unless the potential hazards of NDs to humans and other biological systems are ascertained. Previous studies performed have demonstrated the excellent biocompatibility of NDs in a variety of cell lines without noticeable cytotoxicity. This paper reported the first genotoxicity study on NDs. The results showed that incubation of embryonic stem cells with NDs led to slightly increased expression of DNA repair proteins, such as p53 and MOGG-1. Oxidized NDs (O-NDs) were demonstrated to cause more DNA damage than the pristine/raw NDs (R-NDs), showing the surface chemistry specific genotoxicity. However, the DNA damages caused by either the O-NDs or the R-NDs are much less severe than those caused by multiwalled carbon nanotubes (MWNTs) observed. These findings should have important implications for future applications of NDs in biological applications *(Xing et al., 2011).*

CONCLUSIONS

The unique ND properties have demonstrated exceptional performance in various fields. ND is the state-of-the-art material widely used in polishing materials, polymers, lubricants and electrolytes. ND is rapidly finding its way into biotechnologies, thermal management in microelectronics, advanced composite materials, field emission displays, and drug delivering and targeting carriers and in other 21st century applications. Unfortunately, there is still an important factor that limits the application of nanodiamond as a potential drug delivery tool. The cytotoxicity of ND need to be further investigated, especially the long-term effects on cell or an animal.

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