Expert Opinion

Nanodiamonds as vehicles for systemic and localized drug delivery

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Owing to their exceptional biocompatibility and unique surface properties, nanodiamonds (NDs) are shown to be a progressively promising nanomaterial for drug delivery. In this article, NDs as a platform for a host of biomedical applications are described, with an emphasis on cancer therapy, ranging from systemic modalities to primary constituents within polymer hybrid microfilms. Experimental results and theoretical explanations of ND–drug dynamics are compared. Water-dispersion of previously insoluble therapeutics when complexed with NDs demonstrates great promise in expanding current drug delivery options. Various forms of incorporating NDs within microfilms as a localized drug release coating and implant are also discussed.

Keywords: cancer, drug delivery, imaging, nanodiamond, targeting

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1. Introduction

The burgeoning field of nanoparticle-based drug delivery systems aims to improve on or possibly replace existing drug administration methods. Towards this end, nanoparticles are being pursued as future comprehensive treatments, proposed as being utilized anywhere from detection to diagnosis to treatment [1,2]. Several crucial factors have been historically considered when selecting a drug delivery platform. Mounting evidence has indicated that drug efficacy is determined through a combination of factors, including, among others, the timing of drug administration, dosages and drug release patterns [3,4].

Nanomaterials in particular can play a key role in addressing these issues, as their size potentially allows for unprecedented treatments (i.e., by means of signaling pathway regulation) and post-therapeutic response. In the instance of tumor penetration, the measured vascular permeability pore cutoff size is anywhere from 200 nm to 1.2 µm, depending on the type of tumor and microenvironment [5,6]. Therefore, in order to have access to all regions within varying tumors, the drug carrier and agent should ideally be on the nanoscale. A range of reports has supported this theory, as nanoparticles have shown that existing chemotherapeutic efficacy can be improved on in addition to a decrease in side effects [7].

In order for nanomaterials to be a clinically viable option, however, several issues must first be addressed. In this review, the appeasement of these issues through a variety of studies with nanodiamonds (NDs) is discussed.

2. Background and properties

First, in order for any material to be considered, it must be easily mass produced. Since their discovery and inception 45 years ago, NDs have been synthesized in a variety of ways [8,9]. In this respect, NDs are favorable as they can be scalably produced via processes that are easily optimized through batch ultrasonication,

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ball milling and acid washing that yield individual impurity-free particles of 4 – 6 nm diameter [10-13]. In this work, a few properties are highlighted that make NDs promising for drug delivery applications.

NDs, typically modeled as truncated octahedrons, have an aspect ratio of near unity, which bears semblance to other well-researched drug carriers, such as micelles, liposomes, double emulsions and other spherical nanoparticles [14-19]. Other drug delivery carriers with higher aspect ratios, such as carbon nanotubes [20] and worm micelles [21], have been investigated owing to different interesting properties in cell penetration and flexibility. Still other carbon-based nanomaterials have been reported for their unique drug delivery abilities as well [22,23].

NDs show rapid cell membrane translocation for active drug delivery and biolabeling [24-27]. Very recently, the use of NDs for biomedical imaging applications has been initiated because of their unique fluorescing properties in addition to their presumed biocompatibility, a feature not easily conferred to quantum dots. In conjunction with their biocompatibility and drug delivery capabilities, the fluorescent properties of NDs have resulted in their mass appeal as a multifaceted imaging agent.

A variety of methods have used NDs as biological imaging agents. Nitrogen-vacancy center defects created through various types of irradiation on 35 and 100 nm NDs have generated photostable fluorescence excitation and emission at ~ 560 and ~ 680 – 700 nm, capable of high spatial and temporal resolution within cells [28-30]. Advances in defect generation have made mass-production of these fluorescent particles possible [29]. An added benefit in this method of production is that the fluorescence originates from point defects within the lattice, and therefore is uninhibited by surface functionalization. A preliminary study demonstrated that DNA could nonspecifically attach onto carboxylated NDs without any loss in fluorescence [30]. Mochalin and Gogotsi demonstrated an interesting alternative in creating fluorescent 5 nm NDs [31]. Instead of using irradiation, the authors covalently bound octadecylamine (ODA) to the surface, finally generating blue fluorescent NDs that were soluble in a variety of hydrophobic solvents [31].

Another contrasting method in using NDs as biolabeling agents is through Raman spectroscopy. Diamond materials have a strong Raman intensity signal at 1332 cm^{-1} due to its sp^3 carbon bonds, a wavelength that is typically independent of surface functionalization and biomolecules [32,33]. Furthermore, Raman spectroscopy is performed at ambient conditions and does not invasively harm the cell. One hundred-nanometer-diameter carboxylated NDs conjugated with growth hormones were used to label growth hormone receptors within epithelial cells and identified through its unique Raman signature [34]. Potential future work manipulating the NDs may demonstrate further their application as both a simultaneous imaging and drug release platform with preclinical and clinical relevance.

Proven to be a versatile platform, NDs have also been conjugated and attached to several types of biologically relevant agent, including amino acids and peptides through silane linkage [35], and cytochrome *c* and lysozymes adsorbed by means of surface–protein electrostatic interactions [32,36]. In addition, NDs have been functionalized to capture glycoproteins within protein mixtures, a promising precursor for extraction in proteomics [37]. Future conjugations are very plausible, as diamond surfaces have been conjugated with a variety of extra agents. DNA has been covalently immobilized on both diamond powders of 1 – 2 μm diameter and ND thin films by means of covalent linkages [38,39]. Protein-antigens (mussel adhesive protein [MAP]) have been shown to have conformation stabilization on large (5 – 300 nm) ND particles [40]. Furthermore, ND films have also utilized diamond chemistry and properties for use as biosensors [39,41,42]. For more information, comprehensive reviews on ND functionalization and conjugation have been published previously elsewhere [43,44].

Most importantly, the biocompatibility of NDs has thus far proved extremely promising and must continue to be evaluated *in vivo* [45]. An interesting analogue, although acutely promising for drug delivery applications, continued widespread investigations pertaining to the toxicity of carbon nanotubes are being conducted to assess their potential for its clinical translation [46-51]. Similarly, the innate biocompatibility of particulate NDs has been tested extensively [45]. *In vitro* studies have included cell viability assays, such as mitonchondrial function (MTT) and luminescent ATP production across a variety of cell lines [52]. Comparisons between the biocompatibility of carbon nanotubes and NDs have been examined directly within *in vitro* assays by means of MTT assays, mitochondrial membrane permeability and the generation of reactive oxygen species (ROS) across A459 lung epithelial cells [53] and across neuroblastoma and lung alveolar macrophage cell lines, with favorable results for the latter [54]. Diamond powders have been shown to possess great compatibility *in vitro* and *in vivo*, with the possibility of lipid peroxidation inhibition in blood plasma *in vitro* and an absence of body reaction *in vivo* [55]. Recent work has investigated the biocompatibility of NDs at the genetic level by means of real-time quantitative polymerase chain reactions (RT-PCR), evaluating a variety of inflammatory cytokine responses on ND incubation [56]. Genes associated with inflammation (interleukin-6, tumor necrosis factor-α, inducible nitric oxide synthase) and apoptotic behavior (Bcl-x) did not show any discernible discrepancy in expression versus normal controls. These results have suggested that the mass, purity, aspect ratio and surface functionalization all contribute highly to the biocompatibility of carbon-based nanomaterials [54].

In addition to this, it has been strongly suggested that ND colloidal stability and dispersibility within solutions significantly influences biocompatibility [57]. Steady progressions in creating stable suspensions of NDs within aqueous media have thus steadily improved and could be ready for translation to biomedical applications [11,58,59].

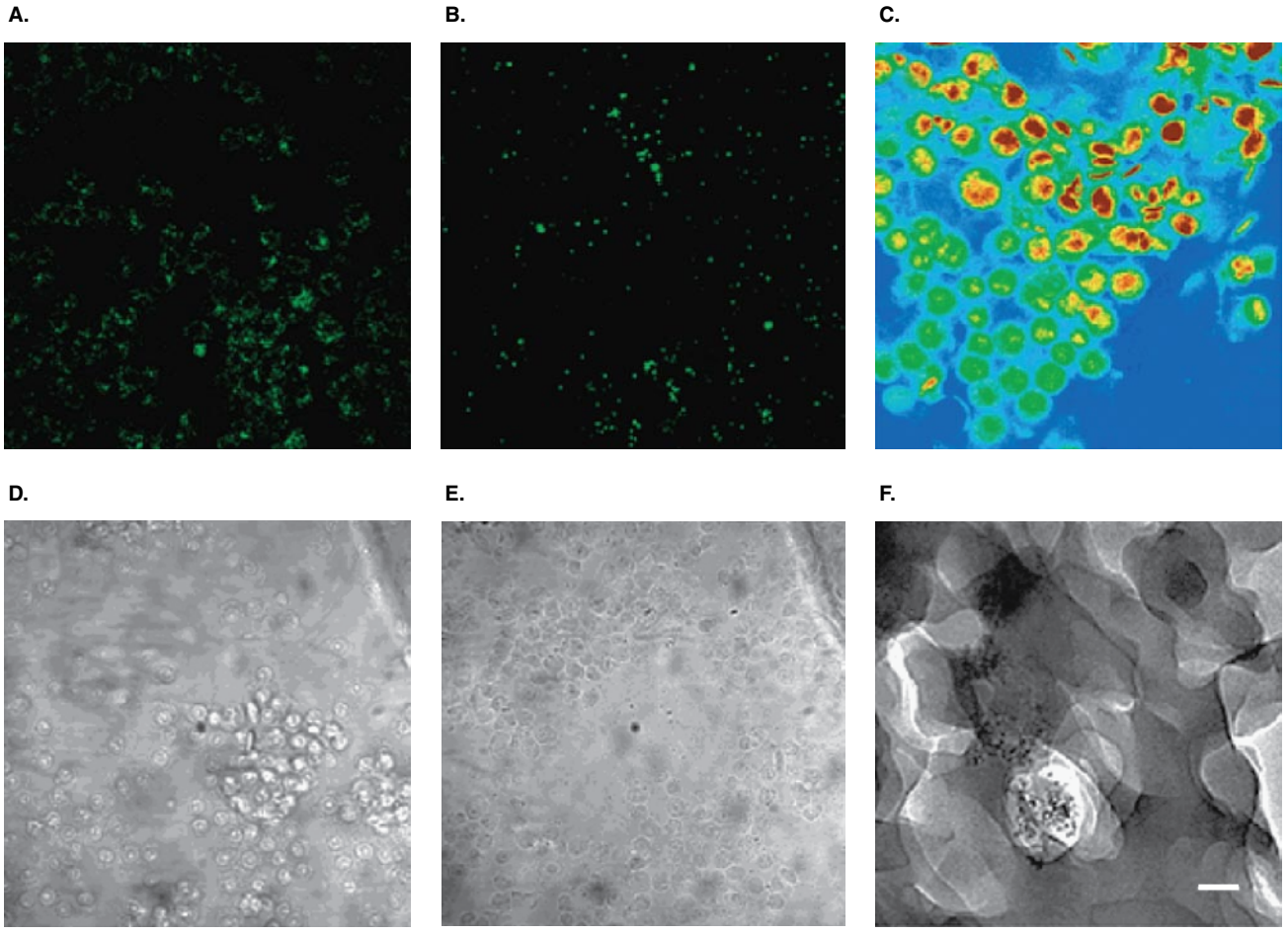

Figure 1. Nanodiamonds easily internalized into cells for drug release. A – E. Confocal images of fluorescently (FITC)-labeled nanodiamonds incubated with RAW 264.7 macrophages. **F.** Transmission electron microscope image of ND–DOX complexes within the cytoplasm of macrophage cells. Scale bars represent 20 nm.
Reproduced with permission from [56].

For these aforementioned reasons, NDs are reviewed herein as a basis to form a comprehensive array of both systemic and localized treatments. Newly created ND–drug conjugates have demonstrated promise in not only improving current therapeutic efficacies, but also creating new avenues in delivering previously unrealized drugs. For localized treatments, ND-based films constructed for a variety of treatments and afflictions, including chemotherapy, cardio-thoracic medicine and wound-healing, are reviewed.

3. Towards systemic delivery

For systemic treatments, NDs are visualized as a nanoparticle drug carrier that addresses a multitude of diseases, in particular late stage malignant cancers. Owing to their small size and biologically amenable surface, penetration of leaky vasculature for thorough therapeutic exposure is possible. As opposed to localized implants, the nanoparticles would provide a non-surgical method of introduction. In order for this to be realized, continued material design is being addressed. Nascent phases of administration, tissue penetration, cellular and pharmacological biodistribution would be optimized in order to control the *in vivo* fate of the particles [20]. Key issues addressing clearance times and residual accumulation would also be investigated. In pursuance of these topics, interesting recent preliminary results of the *in vivo* fate of 50-nm-diameter NDs have already been reported [60].

In the unique problem of systemic delivery to tumor cells, further challenges on the cellular and physiological levels must be addressed. These include drug resistance by cells (multi-drug resistance), physiological barriers (pressure, physiochemical etc.), and eventual biodistribution and clearance from the body [7]. In order for this platform to translate successfully to clinical practice, stringent control over basic properties (i.e., drug functionalization, preservation of drug functionality) would be necessary.

3.1 Nanodiamonds for chemotherapeutic delivery

An initial foray into investigating the capabilities of NDs as promising and cellularly internalized drug carriers was demonstrated with doxorubicin hydrochloride (DOX), a clinically relevant therapeutic for the treatment of a wide array of cancers [56]. DOX was either coated and/or entrapped between ND aggregates, showing a capacity for therapeutic sequestration and reduction of systemic overexposure. As such, because several chemotherapeutics provide biological activity against both healthy and cancerous cells, this ND-mediated 'shielding' effect provides innate protection against nonspecific and unwanted processes. Current clinically offered alternatives, such as liposome packaged doxorubicin (Caelyx®; Schering-Plough, Kenilworth, NJ, Myocet®; Sopherion Therapeutics Princeton, NJ) demonstrate this effect by successfully reducing side effects, such as alopecia and nausea [61,62].

The reversible adsorption and desorption of DOX to and from the ND surface was also shown to be dependent on surface charge or, more generally, pH. Similar effects were demonstrated with water-solubilized carbon nanotubes [23] and amphiphilic copolymer membrane nanocontainers [63]. DOX–ND interactions generated attenuated cell death, with longer incubations resulting in the advancement of apoptotic behavior, further attesting to the ability of NDs as a platform for long-term drug storage and slow release. These results implied ND-mediated preservation of drug functionality, a promising precursor for advanced clinical translation of intelligent drug carriers.

ND-conjugated cell internalization was visualized by means of fluorescence microscopy and transmission electron microscopy (TEM), showing that ND–drug complexes easily traverse the cell membrane for efficient release (**Figure 1**). Both the efficient and the smart delivery capabilities of NDs should be explored further using NDs in a variety of biomedical applications. Owing to recent advances in deaggregation and long-term ND colloidal solution suspension within aqueous media [64-66], these possibilities are now conceivable in a clinical setting.

3.2 Nanodiamond-bioagent dynamics

Owing to recent insights by Barnard and Sternberg [67,68] and further elaboration by Osawa *et al.* [69], a model for electrostatic interactions between the surface of NDs and DOX has been proposed. Through density functional tight binding-based simulations, ND particle–particle interactions demonstrated dependency on the type of surface facet, possibly leading to preferentially ordered self-assembled ND agglutinates (**Figure 2**) [67,68]. For example, (100) surface and (100)/(111) edges featured positive potentials, whereas (111) surfaces contained either extremely negative or varying potentials owing to asymmetry [67]. These effects become important during the process of ND–drug conjugation because the availability of functional groups can potentially be correlated to the reactivity of the various nanodiamond surface planes. The accessibility of functional groups (e.g., -COOH), as well as the absence of functional groups in certain cases, can mediate the opportunities to link various types of therapeutics to the ND surface via several scenarios, which include physisorption and covalent conjugation, for example. Furthermore, the surface reactivity is expected to be impacted further by the composition of the surrounding environments, which can include saline, nanopure water, blood (*in vitro*/*in vivo*), and so on.

This theoretical model provided a basis for describing the interplay between ions, small and water molecules with ND surface facets [69]. Owing to stronger interactions, charged molecules such as salts or certain drugs will competitively adhere to the ND surface, displacing previously adhered water molecules. Depending on the charge, these charged entities will preferentially attach to certain facets or edges. As was demonstrated experimentally and independently previously in [56], this equilibrium between charge–charge and charge–dipole interactions can be shifted according to the number of ions present [69].

In other words, as ever-increasing amounts of drug are attached to the ND surface, the surrounding water molecules are displaced further and the ND–drug complex's solubility decreased [69]. This model is summarized in general as:

$$D_n + (H_2O)/F \rightleftharpoons (D_n)/F + H_2O$$
$$(D_n)/F \rightleftharpoons (D)/F + D_{n-1}.$$

where D_n is the drug agglomerate, D is the dispersed polar drug molecule, F is the facet and '/' symbolizes the interaction [69].

3.3 ND dispersal of water-insoluble therapeutics

The aforementioned dynamic between therapeutics and NDs led to surprising results and exciting behaviors. Traditionally, systemically administered drugs have been limited by aqueous solubility [70]. This can be especially challenging for small molecules of poor water dispersion. To address this challenge successfully, a new report has demonstrated the water solubilization of previously water-insoluble therapeutic compounds, through ND cluster–drug interactions [71].

Specifically, Purvalanol A and 4-Hydroxytamoxifen (4-OHT), promising drugs for liver and breast cancer, respectively, and the anti-inflammatory Dexamethasone, were complexed with ND clusters, greatly improving water dispersion. The ability to deliver water-insoluble therapeutics has also been addressed using other carbon-based nanomaterials where functionalized graphene oxide sheets were shown to attach to insoluble drugs and render them stable within aqueous and biological solutions [22]. A similar effect can be shown by entrapping hydrophobic molecules within triblock copolymer-based nanospheres or worm micelles [21,72].

Purvalanol A and 4-OHT are typically solubilized in DMSO or ethanol, respectively, solvents normally unsuitable or unfavorable for injection. Systemic administration of 4-OHT thus far has been shown to reduce estrogen receptor-positive breast cancer, but unfortunately increased the risk of thromboembolism and endometrial cancer [73,74].

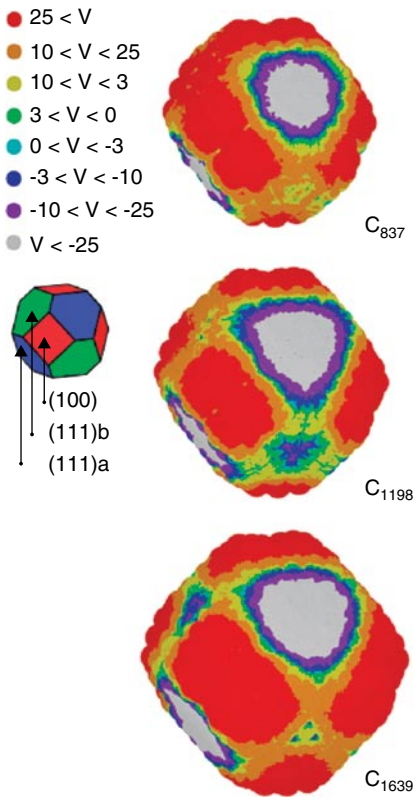

Figure 2. Different facets on nanodiamond surfaces have varying electrostatic potentials. The models depict distinct potentials dependent on the type of surface. Note that facet edges have dramatically different potentials from facet faces.
Reproduced with permission from [68].

Before ND-complexing, Purvalanol A and 4-OHT aggregated into particles on the order of ~ 100 µm. Subsequent binding with NDs resulted in a 3 orders of magnitude decrease in particle size on the order of ~ 100 nm. In addition, the zeta-potential of the resulting drug–ND complexes emerged more positive, augmenting the surrounding of hydration shells, ultimately increasing water dispersibility [71]. These findings are of particular promise, as it has been shown that decreased particle size along with a positively charged surface allow for enhanced cellular internalization [75,76]. Accordingly, NDs have been proposed as a probable candidate for breast cancer pharmacotherapy owing to ND retention in cytoplasmic vesicles within estrogen receptor-positive breast cancer cells (MCF-7) [24]. These effects, in addition to the biocompatibility, scalability and versatility of NDs, lend themselves well as an important precursor for emerging water-insoluble therapeutic delivery.

3.4 A new method for nanodiamond and cell response characterization

To realize the potential in using NDs for systemic delivery, the mechanisms of cellular uptake and intracellular trafficking must be investigated. So far, it is known that pH and salts within buffers have a considerable effect on ND aggregation and stability, which ultimately affects cell internalization behavior [27]. As an alternative means to study this at the single cell level, nanofountain probes (NFPs), normally used in ultra-high-resolution pattern studies, were instead leveraged for cellular injection [77-79]. Fluorescently tagged NDs were injected into a variety of cell lines by means of capillary forces (**Figure 3**). As physical mechanisms were used to introduce the nanoparticles into the cell, no chemical modifications on the probe or ND surface were required during the introduction process.

Experiments regarding cell response and dosage can be explored for further optimization of properties beneficial to systemic delivery. More information is needed for intracellular dynamics of NDs within cells in order to optimize properly the particles as drug carriers. As an inductive study, from injection, it was measured that the diffusion coefficient of 4 – 6 nm NDs was $(11.8 \pm 0.2) \times 10^{-3}$ µm^2/s for the first ~ 5 min, in comparison with a previously reported value of 3.1×10^{-3} µm^2/s for 35 nm NDs [29,78].

The interplay between cellular response and size has shown that particle properties have a crucial role in mediating cellular response [80]. As the NFPs are operated with an atomic force microscope (AFM), real-time control over spatial fidelity and measurement of forces can be made on the order of nanometers and nanonewtons, respectively. Investigations comparing the kinetics of transmembrane transported and intracellular injected NDs within cells are underway at present. The resulting findings could provide a basis in determining optimal dosages and timing sequences in more complex situations.

NFPs were also used simultaneously for high-resolution nanomanufacturing, with nanoparticle placement on the order of 100 nm. Previous ND patterning techniques have demonstrated increased spatial fidelity of ND positioning on larger scales. For example, very recently, inkjet printing techniques with patterned ND solution features on the order of 10 – 100 µm were achieved [81,82]. Intended for CVD templating purposes, the parallel and ambient processing technique would feasibly be well suited for biomedical applications as well.

The current NFP capability reveals the potential for future studies involving single-cell dosage assays and the manufacturing of intelligent devices that require fine consistent dosage control. Therefore, the authors believe this work provides a simple system in which to test for drug delivery efficacy, timescales and dosages for new nanoparticle delivery mechanisms.

4. Towards localized delivery

Numerous challenges are presented when designing biomaterials for drug delivery. Among the most important for implantable drug delivery systems are the need for improved biocompatibility, a reduction in dimensional penalties,

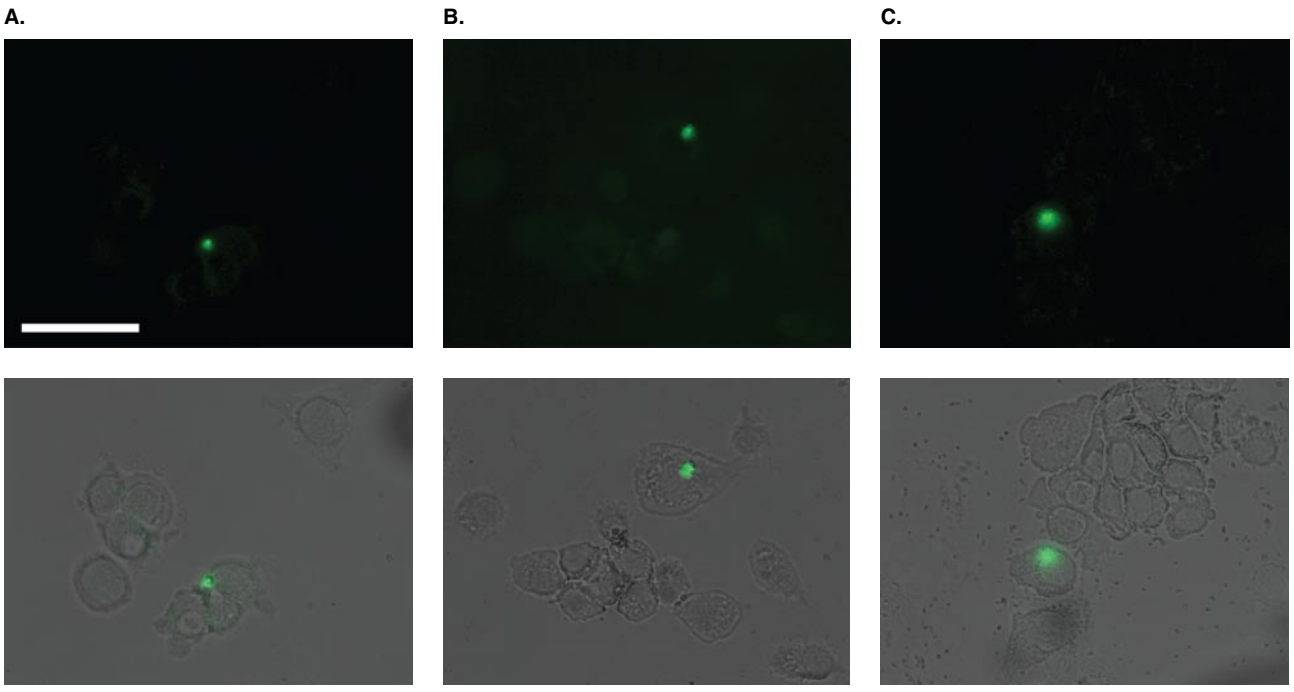

Figure 3. Nanofountain probe injection of fluorescently labeled nanodiamonds in (A) MCF-7 human breast adenocarcinomas, (B) RAW 264.7 murine macrophages and (C) RKO colorectal carcinomas verified by means of epifluorescence (top row) and fluorescence-bright field overlay (bottom row) images. Scale bar is 50 μm.
Reproduced with permission from [78].

tunability of release rates, reduction in nonspecific elution, inhibition of over-elution or 'burst' release, scalability of material production, and evaluation of post drug release effects [83,84]. Focusing the delivery of drugs to one area will cause a reduction of overall systemic drug concentrations. By combining this with preservation of drug activity during sequestration, multiple follow-up therapies could be alleviated or even eliminated [4]. For example, localized delivery of chemotherapeutic has also been shown to reduce systemic side effects, while simultaneously improving drug efficacy [85]. Examples of previously demonstrated long-term delivery methods include modifications of diffusion-based systems and microchip deliveries [86,87]. Herein, facile ND-based strategies capable of controllable long-term drug release, without the need for external energy sources or complex equipment, are discussed.

4.1 Current challenges in sustained and localized chemotherapeutic delivery

Typically, the duration and scope of chemotherapy are restricted to the inherent toxicity effects of the drug within other organs. *In vivo* studies comprised of normal DOX administration implicated several problems associated with drug resistance. Owing to low diffusion rates and strong intracellular binding, large therapeutic gradients were identified immediately after injection that eventually lingered near blood vessels, leaving cancerous cells further removed from vessels unharmed [88]. The same study showed that continuous infusion administration led to more graduate gradients [88]. Multicellular layer models have shown that for several drugs, slowed penetration effectively combats chemotherapy efficacy, especially for cells distant from blood vessels [89]. Sequential treatments are highly considered to remove successive layers of cells on treatment, both on the periphery and near blood vessels [89].

Therefore, by having perpetual and localized release, problems normally associated with drug penetration can be bypassed [90,91]. Within spheroid studies, DOX concentrations have been increased within cells situated away from the periphery through continuous infusion, strongly implying improved retention and therapeutic equilibration across the entire tumor [92]. In other words, in order to access cancerous cells that are receiving diminished nutrients, energy and therapeutic, long-term strategies that remove subsequent layers of cells will finally allow access to previously elusive cells. Advanced *in vitro* and *in vivo* models for studying drug penetration would generate helpful supporting insights [93].

In lieu of these effects, the design of a localized drug delivery system should provide a basis for controlling dosages over a long period of time, as well as maintaining a platform for improved biocompatibility and versatility.

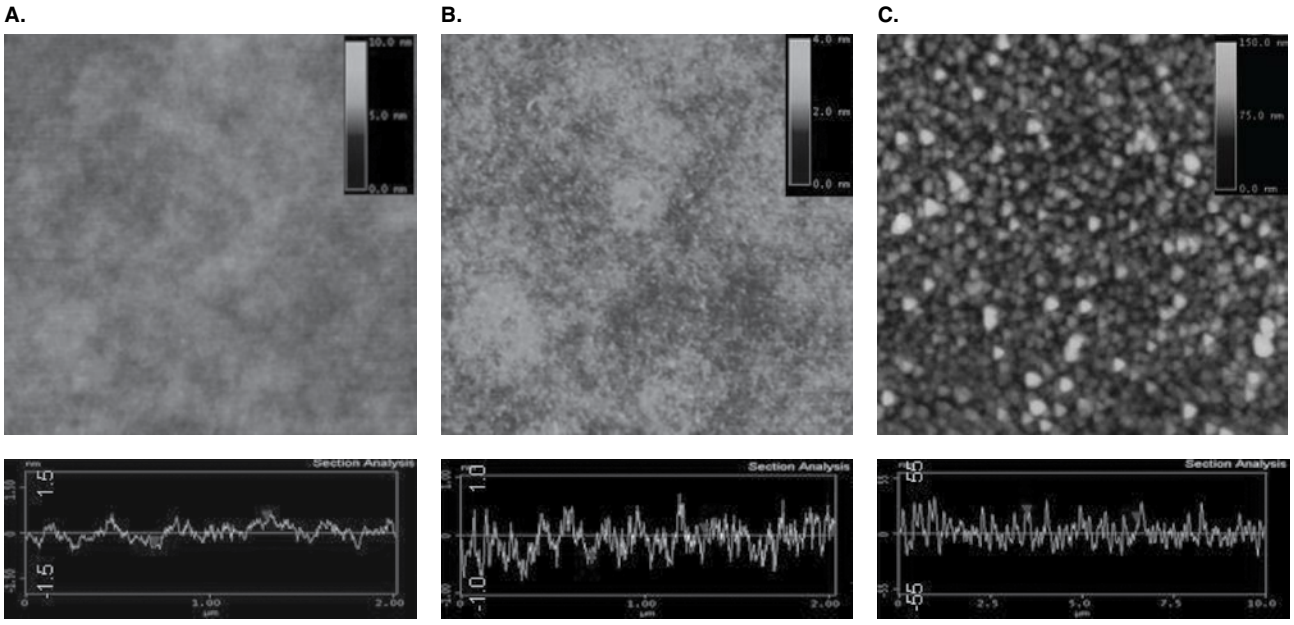

Figure 4. Layer-by-layer deposition process: sequential 2 × 2 µm AFM scans of (A) glass, (B) poly-l-lysine and (C) ND thin film layer.
Reproduced with permission from [94].

4.2 Biocompatible and biofunctional multilayer nanodiamond films

An interesting application transferring the nanoscale properties of NDs to the macroscale was demonstrated by forming template ND nanofilms through layer-by-layer (LBL) deposition [94]. Routes towards future biosensor applications have also been demonstrated where NDs were coated onto a glass surface through a silane coupling agent [95].

The concept of ND-based localized therapy bioimplantation was developed further through LBL processes with proteins. Several aspects of this mode of fabrication, utilizing the sequential adsorption of polyanions and polycations, lend themselves as a prevalent utility for biomedical applications. These include the ease of use under the allowance of a variety of substrates and respective surface topographies, integrated control over composition and thickness, and fabrication under ambient conditions, among others [96,97]. Of particular interest are the removed need for expensive and extreme chemical vapor deposition equipment and conditions, the ease in which nanofilm thickness can be controlled, and the allowance and control over an amenable material–tissue interface forges a favorable method for coating implants of assorted shapes and sizes [97].

Owing to the abundance of -OH and -COOH functional groups on the ND surface, simultaneously allowing for water dispersion and providing a negative surface charge, layers of interspersed ND clusters could be deposited onto positively charged poly-L-lysine (PLL)-coated surfaces (**Figure 4**). The resulting film created interparticle cavities, potentially allowing for drug storage and future release. The resulting LBL-ND films were found to be stable, even when submerged within salt solutions, a testament to the strong electrostatic and van der Waals interactions between the PLL substrate and ND layer [94].

Several biocompatibility assays authenticated the retained biocompatibility of the ND films, showing similar profiles down to the genetic level as their dispersed brethren along with standard negative controls. Preliminary studies integrating the films with anti-inflammatory glucocorticoid steroid hormones (Dexamethasone) by means of physical interactions showed suppressed expression of inflammatory cytokines as a result of sustained therapeutic release [94].

As the amount of ND layers can be controlled and measured easily, both drug adsorption amounts and desorption rates can potentially be controlled, allowing for future applications in a variety of implantation coatings, or if made more robustly, used as a standalone drug release strategy. This finding makes it favorable for other fields of interest, such as anticancer and gene delivery applications by means of controlled delivery of cytotoxic chemicals or DNA, respectively.

An assortment of biologically relevant agents could be assimilated into LBL films by changing the ND surface chemistry, such as reacting amino-reactive sites on a molecule to an amine functionalized ND surface. Finally, past works using various stimuli (pH-, thermo- etc.) to trigger release within LBL films also provide further avenues of inquiry [97].

4.3 Hybrid nanodiamond-polymer microfilms for localized chemotherapeutic delivery

Another method for translating the aforementioned ND-mediated slow-release capabilities to the macroscale is through the

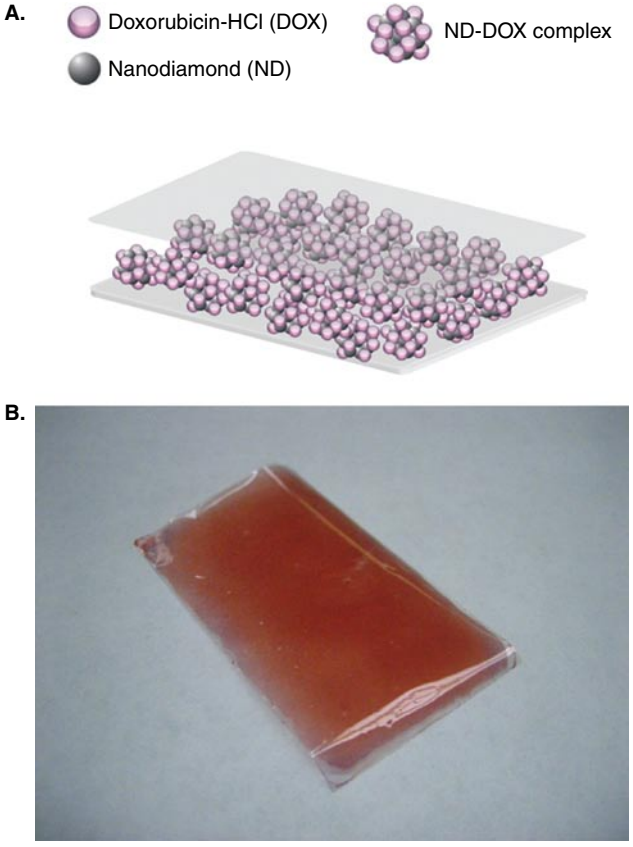

Figure 5. A. Schematic depiction of the construction of nanodiamond-parylene microfilms. Nanodiamonds are sandwiched between a thick base layer and thin variable non-conformal layer of parylene, which allows for controllable release. **B.** Resultant microfilms can be of varied size and are flexible.

embedding of NDs within polymer matrices [98]. Interesting studies using NDs as a constituent within other hybrid materials have demonstrated the versatility in improving various physical properties. For example, the dispersion of NDs within polyacrylonitrile and polyamide by means of electrospinning into microfibers and nanofibers has exciting applications in transparent, scratch-resistant UV protection coatings [99], whereas adding NDs to copper-based nanocomposites has been shown to improve mechanical properties [100].

Parylene C was opted as the structural framework for ND encapsulation. Several properties of this polymer led to its previous use as a coating in FDA-approved devices [101-104]. These include extreme biostability and inertness, standardized conformal coatings even over complex substrates, and tunable thicknesses [102-106]. Traditionally used as a coating, the deposition process of parylene C is also proficient in creating flexible standalone devices of tunable dimensions and shapes.

In constructing this device, ND–DOX complexes were lodged between a thick impermeable base and thin pervious layer of parylene C (**Figure 5**). This design scheme allows for unidirectional drug release originating from ND–drug complexes. Before this, this architectural motif had been explicated without NDs [107-109]. In these studies, the release of Dox was extended from minutes and hours to on the order of days [107,108], implying, through careful determination of the top layer thickness and permeability, drug release can be adjusted accordingly.

The addition of NDs as a constituent not only extended release in a continuous and consistent manner for nearly a month, but also suppressed burst release [98]. There are several advantages to this simple design: the elimination of a need for an energy source or pump to control release while the NDs provide a stable reservoir of drug. Accordingly, the addition to NDs caused lower therapeutic release in spite of having a large amount of drug situated within the microfilm, extending the active lifetime of the film [108].

Designed primarily as an adjuvant therapy for early-stage post-tumor removal implantation, the device is envisioned to minimize dramatically or even eliminate follow-up radiation therapy via potent sustained release and localized chemotherapeutic activity. If successful, significantly less drug could be used, simultaneously improving patient treatment with potentially fewer side effects and abolishing the need for further surgery.

By altering ND conjugation with various disorder-specific agents, the broad applicability of this device is extended, with connotations not only within oncological treatment, but also within the confines of anti-inflammatory remedies, or any purpose that stresses direct localized placement over an afflicted area. As examples, separate constructed microfilms consisting of ND–DOX [107] and 4OHT conjugates (Robert Lam, Dean Ho, unpublished results, 2009) corroborated the inclusive nature of this design. Future iterations expounding on the potential of this device include using microfabrication techniques for precise drug placement [87,110] and delivery, the addition of combinatorial therapeutics [111] and imaging agents to create an all-encompassing device.

5. Conclusion

The role of utilizing NDs within drug delivery applications is still in its infancy. As a result, several tasks are needed to characterize fully the nature of NDs both physically and within a biological setting. A clinically applicable platform strategy will require further biocompatibility tests, optimization of ND–drug conjugation and strict control over key physical parameters. Within these aspects, initial results in biocompatibility and efficacy have been promising thus far. New methods of cell reaction interrogation could provide a quick methodology in identifying intracellular responses and mechanisms. For localized delivery systems, NDs could become key contributors in providing sustained release and improving biocompatibility as a coating or standalone drug release device owing to their flexibility and ease of manufacture.

6. Expert opinion

The continued search for the optimized design of a versatile and efficient drug carrier is catalyzing multidisciplinary efforts that integrate the fields of materials science, bio/medical engineering, chemistry, biology, toxicology, and beyond. With respect to ND-based carriers, *in vivo* testing of both safety and efficacy of therapeutic delivery or imaging will be examined towards the realization of their translational relevance. As a means to bridge the gap from *in vitro* to *in vivo* biocompatibility studies, three-dimensional cell-cultured-based toxicity studies could be performed [112]. Drastic differences in toxicity when comparing nanoparticles within standard two-dimensional cultures and three-dimensional spheroid cultures, possibly because of the more accurate tissue simulation, could help mediate long-term toxicity studies [112]. For example, *in vitro* multicell-spheroid studies relating DOX gradients with tumor heterogeneity were later verified *in vivo* [88,113]. Using more tumor models both *in vitro* and *in vivo*, multi-therapeutic release, or combinatorial therapy, as well as sequential therapy and the addressing of chemoresistance should also be performed given the well-established findings that multi-agent treatment of diseases can have a profound impact on the improved efficacy of therapeutic activity.

At the foundation of the application-specific development of NDs for therapeutic delivery, further elucidation of the versatility and subsequent tenability of the ND surface will serve as a gateway to pairing modeling and simulation with experimental validation towards intelligent ND platform design. This collaborative effort combining theoretical modeling and simulation-based optimization would help identify key parameters such as the density spatial distribution of chemical group functionalization on the ND surface (e.g., amine, carboxylic acid, hydroxyl group presence). These factors can also influence significantly the extent of drug or imaging/targeting agent loading, and govern delivery rates. Furthermore, the elucidation of the surface composition of NDs can also inspire new conjugation routes, boosting the applicability of the ND platforms towards new therapeutic-diagnostic, or 'theranostic' scenarios. To these ends, one can also gain insight from previous *in vivo* nanoparticle studies. Several examples, including recent insights on systematically PEGylated-near-infrared emitting quantum dots, show that surface charge and size play a major role in *in vivo* biodistribution and clearance [114]. In addition, quantum dots functionalized with ultrashort PEG chains were rapidly absorbed by the liver; longer PEG chains increased retention times and intermediary hydrodynamic diameters displayed various surprisingly specific uptake, targeting and clearance [114]. The large surface area and surface functionalization are key determinants in *in vivo* toxicity [20,115]. Previous work with C_{60} fullerenes has demonstrated drastically different reactions based on surface modifications [116-118].

Further milestones of importance would also include the pursuit of combining targeting, imaging and drug release capabilities of NDs into a single platform with the preservation of the aforementioned biocompatibility (e.g., non-cytotoxic, ability for clearance etc.) attributes in an *in vivo* setting. Although nearly a reality *in vitro*, applicability *in vivo* would prove more complex as NDs would need to be capable of fluorescing in a medically relevant range of wavelengths (i.e., infrared, PET/CT/MRI) in addition to coupling with therapeutics. Coincidentally, Miyawaki *et al.* recently developed a way to measure quantitatively the biodistribution of Gd_2O_3-labeled carbon nanohorns within mice by means of inductively coupled plasma atomic emission spectroscopy (ICP-AES) [119]. Continued work in this area will draw on emerging findings from the aforementioned investigations into ND surface properties to allow for the co-loading of imaging agents (e.g., PET/MRI probes) and therapeutic compounds.

It has been hypothesized that the ND surface can temporarily inactivate bound drug molecules, providing a means for storage of drugs while suppressing side effects [69]. Future studies on the possibility of combining targeting and preservation of therapeutics could signal a transformative effect on systemic timed release. To translate these attributes to localized delivery methods, immobilization of NDs within photopolymerizable and photodegradable gels, for example, could allow for future unrealized avenues of research, impacting and meeting diverse medical needs that range from neural implantation (e.g., controlled release of neural growth factors for spinal cord repair applications) to implant coatings and the suppression of fouling/immunological rejection. Furthermore, enhancements to localized drug delivery can be realized further in the form of new capabilities in temporal and spatial drug release. At the heart of ND design and fundamental evaluation resides the importance of uniting several disciplines (e.g., nanomanufacturing, drug discovery, molecular biology/medicine) that will each play unique as well as collaborative rolls in the downstream realization of clinical ND therapy.

Declaration of interest

The authors state no conflict of interest and have received no payment in the preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Farokhzad OC, Langer R. Impact of nanotechnology on drug delivery. ACS Nano 2009;3(1):16-20
2. Salata OV. Applications of nanoparticles in biology and medicine. J Nanobiotechnol 2004;2(1):3
3. Bakken EE, Heruth K. Temporal control of drugs. Ann NY Acad Sci 1991;618(temporal control of drug delivery):422-7
4. Langer R. New methods of drug delivery. Science 1990;249(4976):1527-33

• **This report discusses important routes towards optimizing nanomaterial-based drug delivery.**

5. Yuan F, Dellian M, Fukumura D, et al. Vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size. Cancer Res 1995;55(17):3752-6
6. Hobbs SK, Monsky WL, Yuan F, et al. Regulation of transport pathways in tumor vessels: role of tumor type and microenvironment. Proc Natl Acad Sci USA 1998;95(8):4607-12
7. Brigger I, Dubernet C, Couvreur P. Nanoparticles in cancer therapy and diagnosis. Adv Drug Deliv Rev 2002;54(5):631-51
8. Danilenko V. On the history of the discovery of nanodiamond synthesis. Phys Solid State 2004;46(4):595-9
9. Dolmatov VY. Detonation synthesis ultradispersed diamonds: properties and applications. Usp Khim 2001;70(7):687-708
10. Huang HJ, Dai LM, Wang DH, et al. Large-scale self-assembly of dispersed nanodiamonds. J Mater Chem 2008;18(12):1347-52
11. Osawa E. Recent progress and perspectives in single-digit nanodiamond. Diamond Relat Mater 2007;16(12):2018-22
12. Osawa E. Disintegration and purification of crude aggregates of detonation nanodiamond. Synth Properties Appl Ultrananocrystalline Diamond 2005;192:231-40

•• **This paper describes the processing of detonation nanodiamonds from aggregate to single particle form, providing an important foundation towards enhancing the biomedical relevance of nanodiamonds with respects to functionalization with bioactive compounds and biocompatibility.**

13. Krueger A. The structure and reactivity of nanoscale diamond. J Mater Chem 2008;18(13):1485-92
14. Lian T, Ho RJY. Trends and developments in liposome drug delivery systems. J Pharm Sci 2001;90(6):667-80
15. Kataoka K, Harada A, Nagasaki Y. Block copolymer micelles for drug delivery: design, characterization and biological significance. Adv Drug Deliv Rev 2001;47(1):113-31
16. Drummond DC, Meyer O, Hong KL, et al. Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. Pharmacol Rev 1999;51(4):691-743
17. Hanson JA, Chang CB, Graves SM, et al. Nanoscale double emulsions stabilized by single-component block copolypeptides. Nature 2008;455(7209):85-8

•• **Double nanoemulsions synthesized in this work demonstrated unprecedented long-term stability and the capacity for simultaneous loading with hydrophilic and hydrophobic compounds.**

18. Paciotti GF, Kingston DGI, Tamarkin L. Colloidal gold nanoparticles: a novel nanoparticle platform for developing multifunctional tumor-targeted drug delivery vectors. Drug Dev Res 2006;67(1):47-54
19. Rosi NL, Giljohann DA, Thaxton CS, et al. Oligonucleotide-modified gold nanoparticles for intracellular gene regulation. Science 2006;312(5776):1027-30

•• **This work demonstrates the application of gold nanoparticles as RNA delivery agents with preserved intracellular capacity for gene regulation.**

20. Lacerda L, Bianco A, Prato M, Kostarelos K. Carbon nanotubes as nanomedicines: from toxicology to pharmacology. Adv Drug Deliv Rev 2006;58(14):1460-70
21. Kim Y, Dalhaimer P, Christian DA, Discher DE. Polymeric worm micelles as nano-carriers for drug delivery. Nanotechnology 2005;7:S484
22. Liu Z, Robinson JT, Sun X, Dai H. PEGylated nanographene oxide for delivery of water-insoluble cancer drugs. J Am Chem Soc 2008;130(33):10876-7

• **Demonstration of nanocarbon-mediated water-insoluble therapeutic delivery.**

23. Liu Z, Sun X, Nakayama-Ratchford N, Dai H. Supramolecular chemistry on water-soluble carbon nanotubes for drug loading and delivery. ACS Nano 2007;1(1):50-6
24. Khan SA, Ivancic DZ, Zaichuk T, et al. Internalization and retention of nanodiamonds by MCF-7 cells in vitro: a potential drug delivery platform. Cancer Res 2009;69(2_MeetingAbstracts):2134
25. Vial S, Mansuy C, Saga S, et al. Peptide-grafted nanodiamonds: preparation, cytotoxicity and uptake in cells. ChemBioChem 2008;9(13):2113-9
26. Faklaris O, Garrot D, Joshi V, et al. Detection of single photoluminescent diamond nanoparticles in cells and study of the internalization pathway. Small 2008;4(12):2236-9
27. Neugart F, Zappe A, Jelezko F, et al. Dynamics of diamond nanoparticles in solution and cells. Nano Lett 2007;7(12):3588-91
28. Yu SJ, Kang MW, Chang HC, et al. Bright fluorescent nanodiamonds: no photobleaching and low cytotoxicity. J Am Chem Soc 2005;127(50):17604-5
29. Chang YR, Lee HY, Chen K, et al. Mass production and dynamic imaging of fluorescent nanodiamonds. Nat Nanotechnol 2008;3(5):284-8

•• **Scalable nanodiamond imaging agent synthesis and intracellular fluorescence activity are demonstrated in this work.**

30. Fu C-C, Lee H-Y, Chen K, et al. Characterization and application of single fluorescent nanodiamonds as cellular biomarkers. Proc Natl Acad Sci 2007;104(3):727-32
31. Mochalin VN, Gogotsi Y. Wet chemistry route to hydrophobic blue fluorescent nanodiamond. J Am Chem Soc 2009;131(13):4594-5

• **This work demonstrates the unique surface and binding properties of the nanodiamond surface for possible biomedical applications.**

32. Chao J-I, Perevedentseva E, Chung P-H, et al. Nanometer-sized diamond particle as a probe for biolabeling. Biophys J 2007;93(6):2199-208
33. Knight DS, White WB. Characterization of diamond films by Raman-spectroscopy. J Mater Res 1989;4(2):385-93

34. Cheng CY, Perevedentseva E, Tu JS, et al. Direct and in vitro observation of growth hormone receptor molecules in A549 human lung epithelial cells by nanodiamond labeling. Appl Phys Lett 2007;90(16):163903-3
• **Targeted fluorescent nanodiamond labeling is demonstrated in this work.**
35. Kruger A, Liang YJ, Jarre G, Stegk J. Surface functionalisation of detonation diamond suitable for biological applications. J Mater Chem 2006;16(24):2322-8
36. Huang LC, Chang HC. Adsorption and immobilization of cytochrome c on nanodiamonds. Langmuir 2004;20(14):5879-84
37. Yeap WS, Tan YY, Loh KP. Using detonation nanodiamond for the specific capture of glycoproteins. Anal Chem 2008;80(12):4659-65
• **Protein interactions with nanodiamonds were demonstrated in this work.**
38. Ushizawa K, Sato Y, Mitsumori T, et al. Covalent immobilization of DNA on diamond and its verification by diffuse reflectance infrared spectroscopy. Chem Phys Lett 2002;351(1-2):105-8
39. Yang WS, Auciello O, Butler JE, et al. DNA-modified nanocrystalline diamond thin-films as stable, biologically active substrates. Nat Mater 2002;1(4):253-7
40. Kossovsky N, Gelman A, Hnatyszyn HJ, et al. Surface-modified diamond nanoparticles as antigen delivery vehicles. Bioconjug Chem 1995;6(5):507-11
41. Huang TS, Tzeng Y, Liu YK, et al. Immobilization of antibodies and bacterial binding on nanodiamond and carbon nanotubes for biosensor applications. Diamond Relat Mater 2004;13(4-8):1098-102
42. Hartl A, Schmich E, Garrido JA, et al. Protein-modified nanocrystalline diamond thin films for biosensor applications. Nat Mater 2004;3(10):736-42
43. Krueger A. New carbon materials: biological applications of functionalized nanodiamond materials. Chemistry 2008;14(5):1382-90
44. Kruger A. Hard and soft: biofunctionalized diamond. Angew Chem-Int Ed 2006;45(39):6426-7
• **Prospects and discussion for the biological functionalization of nanodiamonds are discussed in this work, outlining the potential of nanodiamonds for biologically relevant applications.**
45. Schrand AM, Hens SAC, Shenderova OA. Nanodiamond particles: properties and perspectives for bioapplications. Crit Rev Solid State Mater Sci 2009;34(1):18-74
46. Maynard AD, Baron PA, Foley M, et al. Exposure to carbon nanotube material: aerosol release during the handling of unrefined single-walled carbon nanotube material. J Toxicol Environ Health Pt A 2004;67(1):87-107
47. Warheit DB, Laurence BR, Reed KL, et al. Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats. Toxicol Sci 2004;77(1):117-25
48. Lam C-W, James JT, McCluskey R, Hunter RL. Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. Toxicol Sci 2004;77(1):126-34
49. Muller J, Huaux F, Moreau N, et al. Respiratory toxicity of multi-wall carbon nanotubes. Toxicol Appl Pharmacol 2005;207(3):221-31
50. Singh R, Pantarotto D, Lacerda L, et al. Tissue biodistribution and blood clearance rates of intravenously administered carbon nanotube radiotracers. Proc Natl Acad Sci USA 2006;103(9):3357-62
51. Pantarotto D, Partidos CD, Hoebeke J, et al. Immunization with peptide-functionalized carbon nanotubes enhances virus-specific neutralizing antibody responses. Chem Biol 2003;10(10):961-6
52. Schrand AM, Huang H, Carlson C, et al. Are diamond nanoparticles cytotoxic? J Phys Chem B 2007;111(1):2-7
• **Insight into nanodiamond biocompatibility via MTT assays is provided in this study.**
53. Liu KK, Cheng CL, Chang CC, Chao JI. Biocompatible and detectable carboxylated nanodiamond on human cell. Nanotechnology 2007;18(32):10
54. Schrand AM, Dai L, Schlager JJ, et al. Differential biocompatibility of carbon nanotubes and nanodiamonds. Diamond Relat Mater 2007;16(12):2118-23
55. Bakowicz K, Mitura S. Biocompatibility of NCD. J Wide Bandgap Mater 2002;9:261-72
56. Huang H, Pierstorff E, Osawa E, Ho D. Active nanodiamond hydrogels for chemotherapeutic delivery. Nano Lett 2007;7(11):3305-14
•• **Nanodiamond-mediated functionalization and release of a clinically relevant chemotherapeutic was introduced in this work.**
57. Puzyr AP, Neshumayev DA, Tarskikh SV, et al. Destruction of human blood cells in interaction with detonation nanodiamonds in experiments in vitro. Diamond Relat Mater 2004;13(11-12):2020-3
58. Eidelman ED, Siklitsky VI, Sharonova LV, et al. A stable suspension of single ultrananocrystalline diamond particles. Diamond Relat Mater 2005;14(11-12):1765-9
59. Krueger A, Ozawa M, Jarre G, et al. Deagglomeration and functionalisation of detonation diamond. Phys Status Solidi (a) 2007;204(9):2881-7
60. Yuan Y, Chen Y, Liu J-H, et al. Biodistribution and fate of nanodiamonds in vivo. Diamond Relat Mater 2009;18(1):95-100
61. Ranson MR, Carmichael J, O'Byrne K, et al. Treatment of advanced breast cancer with sterically stabilized liposomal doxorubicin: results of a multicenter phase II trial. J Clin Oncol 1997;15(10):3185-91
62. Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. Science 2004;303(5665):1818-22
63. Broz P, Driamov S, Ziegler J, et al. Toward intelligent nanosize bioreactors: a ph-switchable, channel-equipped, functional polymer nanocontainer. Nano Lett 2006;6(10):2349-53
64. Kruger A, Kataoka F, Ozawa M, et al. Unusually tight aggregation in detonation nanodiamond: identification and disintegration. Carbon 2005;43(8):1722-30
65. Ozawa M, Inaguma M, Takahashi M, et al. Preparation and behavior of brownish, clear nanodiamond colloids. Adv Mater 2007;19(9):1201-6
66. Bondar VS, Puzyr AP. Nanodiamonds for biological investigations. Phys Solid State 2004;46(4):716-9
67. Barnard AS, Sternberg M. Crystallinity and surface electrostatics of diamond

nanocrystals. J Mater Chem 2007;17(45):4811-9

• **Important findings characterizing the surface electrostatics of nanodiamond particles.**

68. Barnard AS. Self-assembly in nanodiamond agglutinates. J Mater Chem 2008;18(34):4038-41

69. Osawa E, Ho D, Huang H, et al. Consequences of strong and diverse electrostatic potential fields on the surface of detonation nanodiamond particles. Diamond Relat Mater 2009;18(5-8):904-9

• **Surface electrostatic properties based on nanodiamond composition is addressed in this work, using drug–nanodiamond interactions as a specific model for validation and discussion.**

70. Myrdal PB, Yalkowsky SH. Solubilization of drugs in aqueous media. In: Swarbrick J, Boylan JC, editors, Encyclopedia of pharmaceutical technology: CRC Press; New York, New York 2002. p. 2458-80

71. Chen M, Pierstorff E, Lam R, et al. Nanodiamond-mediated delivery of water-insoluble therapeutics. ACS Nano 2009;(In press) doi: 10.1021/nn900480m

72. Sheihet L, Dubin RA, Devore D, Kohn J. Hydrophobic drug delivery by self-assembling triblock copolymer-derived nanospheres. Biomacromolecules 2005;6(5):2726-31

73. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for the prevention of breast cancer: current status of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst 2005;97(22):1652-62

74. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst 1998;90(18):1371-88

75. Kircheis R, Wightman L, Wagner E. Design and gene delivery activity of modified polyethylenimines. Adv Drug Deliv Rev 2001;53(3):341-58

76. Bettinger T, Remy J-S, Erbacher P. Size reduction of galactosylated PEI/DNA complexes improves lectin-mediated gene transfer into hepatocytes. Bioconjug Chem 1999;10(4):558-61

77. Kim K-H, Moldovan N, Espinosa Horacio D. A nanofountain probe with sub-100 nm molecular writing resolution13. Small 2005;1(6):632-5

78. Loh O, Lam R, Chen M, et al. Nanofountain-probe-based high-resolution patterning and single-cell injection of functionalized nanodiamonds. Small 2009;(In press) doi: 10.1002/smll.200900361

79. Loh OY, Ho AM, Rim JE, et al. Electric field-induced direct delivery of proteins by a nanofountain probe. Proc Natl Acad Sci 2008;105(43):16438-43

80. Jiang W, Kim BYS, Rutka JT, Chan WCW. Nanoparticle-mediated cellular response is size-dependent. Nat Nano 2008;3(3):145-50

81. Chen Y-C, Tzeng Y, Cheng A-J, et al. Inkjet printing of nanodiamond suspensions in ethylene glycol for CVD growth of patterned diamond structures and practical applications. Diamond Relat Mater 2009;18(2-3):146-50

82. Chen Y-C, Tzeng Y, Davray A, et al. Fabrication of diamond micro-structures by ink-jet printed diamond seeding and microwave plasma assisted chemical vapor deposition. Diamond Relat Mater 2008;17(4-5):722-7

83. Langer R, Tirrell DA. Designing materials for biology and medicine. Nature 2004;428(6982):487-92

84. Huang X, Brazel CS. On the importance and mechanisms of burst release in matrix-controlled drug delivery systems. J Control Release 2001;73(2-3):121-36

85. Moses MA, Brem H, Langer R. Advancing the field of drug delivery: taking aim at cancer. Cancer Cell 2003;4(5):337-41

86. Peng L, Mendelsohn AD, LaTempa TJ, et al. Long-term small molecule and protein elution from TiO2 nanotubes. Nano Lett 2009;9(5):1932-6

87. Santini JT, Richards AC, Scheidt R, et al. Microchips as controlled drug-delivery devices. Angew Chem Int Ed 2000;39(14):2396-407

88. Lankelma J, Dekker H, Luque RF, et al. Doxorubicin gradients in human breast cancer. Clin Cancer Res 1999;5(7):1703-7

89. Tannock IF, Lee CM, Tunggal JK, et al. Limited penetration of anticancer drugs through tumor tissue: a potential cause of resistance of solid tumors to chemotherapy. Clin Cancer Res 2002;8(3):878-84

90. Peer D, Karp JM, Hong S, et al. Nanocarriers as an emerging platform for cancer therapy. Nat Nano 2007;2(12):751-60

•• **A comprehensive review of the challenges associated with clinical translation of nanocarriers.**

91. Adams GP, Schier R, McCall AM, et al. High affinity restricts the localization and tumor penetration of single-chain fv antibody molecules. Cancer Res 2001;61(12):4750-5

92. Durand RE. Slow penetration of anthracyclines into spheroids and tumors: a therapeutic advantage? Cancer Chemother Pharmacol 1990;26(3):198-204

93. Minchinton AI, Tannock IF. Drug penetration in solid tumours. Nat Rev Cancer 2006;6(8):583-92

94. Huang H, Pierstorff E, Osawa E, Ho D. Protein-mediated assembly of nanodiamond hydrogels into a biocompatible and biofunctional multilayer nanofilm. ACS Nano 2008;2(2):203-12

95. Liu Y, Khabashesku VN, Halas NJ. Fluorinated nanodiamond as a wet chemistry precursor for diamond coatings covalently bonded to glass surface. J Am Chem Soc 2005;127(11):3712-3

• **A facile route towards generating stable and functionalized nanodiamond thin films is demonstrated in this work.**

96. Decher G. Fuzzy nanoassemblies: toward layered polymeric multicomposites. Science 1997;277(5330):1232-7

97. Tang Z, Wang Y, Podsiadlo P, Kotov NA. Biomedical applications of layer-by-layer assembly: from biomimetics to tissue engineering. Adv Mater 2006;18(24):3203-24

98. Lam R, Chen M, Pierstorff E, et al. Nanodiamond-embedded microfilm devices for localized chemotherapeutic elution. ACS Nano 2008;2(10):2095-102

• **This work introduced the scalable fabrication of nanodiamond-based drug delivery devices with demonstrated long-term release as well as suppression of burst chemotherapeutic delivery.**

99. Behler KD, Stravato A, Mochalin V, et al. Nanodiamond-polymer composite fibers and coatings. ACS Nano 2009;3(2):363-9

100. Hanada K, Yamamoto K, Taguchi T, et al. Further studies on copper nanocomposite

with dispersed single-digit-nanodiamond particles. Diamond Relat Mater 2007;16(12):2054-7

101. Yamagishi FG. Investigation of plasma-polymerized films as primers for parylene-c coatings on neural coatings on neural prosthesis materials. Thin Solid Films 1991;202(1):39-50
102. Schmidt EM, McIntosh JS, Bak MJ. Long-term implants of parylene-c coated microelectrodes. Med Biol Eng Comput 1988;26(1):96-101
103. Hahn AW, York DH, Nichols MF, et al. Biocompatibility of glow-discharge-polymerized films and vacuum-deposited parylene. Appl Polym Symp 1984;38:55-64
104. Chang TY, Yadav VG, De Leo S, et al. Cell and protein compatibility of parylene-C surfaces. Langmuir 2007;23(23):11718-25
105. Burkel WE, Kahn RH. Cell-lined, nonwoven microfiber scaffolds as a blood interface. Ann NY Acad Sci 1977;283(FEB10):419-37
106. Fortin JB, Lu TM. A model for the chemical vapor deposition of poly(para-xylylene) (parylene) thin films. Chem Mat 2002;14(5):1945-9
107. Pierstorff E, Lam R, Ho D. Nanoscale architectural tuning of parylene patch devices to control therapeutic release rates. Nanotechnology 2008;44:445104
108. Robinson EM, Lam R, Pierstorff ED, Ho D. Localized therapeutic release via an amine-functionalized poly-p-xylene microfilm device. J Phys Chem B 2008;112(37):11451-5
109. Chen M, Huang H, Pierstorff E, et al. Parylene-encapsulated copolymeric membranes as localized and sustained drug delivery platforms. Ann Biomed Eng 2009;(In press) doi: 10.1007/s10439-009-9662-9
110. Meng E, Yu-Chong T. Parylene etching techniques for microfluidics and bioMEMS. Micro Electro Mechanical Systems, 2005 MEMS 2005 18th IEEE International Conference on 2005. 568-71
111. Wong PK, Yu F, Shahangian A, et al. Closed-loop control of cellular functions using combinatory drugs guided by a stochastic search algorithm. Proc Natl Acad Sci 2008;105(13):5105-10
112. Lee J, Lilly GD, Doly RC, et al. In vitro toxicity testing of nanoparticles in 3D cell culture. Small 2009;5(10):1213-21
113. Sutherland RM. Cell and environment interactions in tumor microregions: the multicell spheroid model. Science 1988;240(4849):177-84
114. Choi HS, Ipe BI, Misra P, et al. Tissue- and organ-selective biodistribution of NIR fluorescent quantum dots. Nano Lett 2009;9(6):2354-9
115. Oberdorster G, Stone V, Donaldson K. Toxicology of nanoparticles: a historical perspective. Nanotoxicology 2007;1(1):2-25
116. Lyon DY, Alvarez PJJ. Fullerene water suspension (nC(60)) exerts antibacterial effects via ROS-independent protein oxidation. Environ Sci Technol 2008;42(21):8127-32
117. Cagle DW, Kennel SJ, Mirzadeh S, et al. In vivo studies of fullerene-based materials using endohedral metallofullerene radiotracers. Proc Natl Acad Sci USA 1999;96(9):5182-7
118. Yamago S, Tokuyama H, Nakamura E, et al. In vivo biological behavior of a water-miscible fullerene: 14C labeling, absorption, distribution, excretion and acute toxicity. Chem Biol 1995;2(6):385-9
119. Miyawaki J, Matsumura S, Yuge R, et al. Biodistribution and ultrastructural localization of single-walled carbon nanohorns determined in vivo with embedded Gd2O3 labels. ACS Nano 2009;3(6):1399-406

- **Biodistribution and accumulation of carbon nanomaterials *in vivo* are studied in this work, providing a foundation for continued biocompatibility analysis.**

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