Nanodiamonds for In Vivo Applications

Kiran J. van der Laan, Masoumeh Hasani, Tingting Zheng, and Romana Schirhagl

Due to their unique optical properties, diamonds are the most valued gemstones. However, beyond the sparkle, diamonds have a number of unique properties. Their extreme hardness gives them outstanding performance as abrasives and cutting tools. Similar to many materials, their nanometer-sized form has yet other unique properties. Nanodiamonds are very inert but still can be functionalized on the surface. Additionally, they can be made in very small sizes and a narrow size distribution. Nanodiamonds can also host very stable fluorescent defects. Since they are protected in the crystal lattice, they never bleach. These defects can also be utilized for nanoscale sensing since they change their optical properties, for example, based on temperature or magnetic fields in their surroundings. In this Review, in vivo applications are focused upon. To this end, how different diamond materials are made and how this affects their properties are discussed first. Next, in vivo biocompatibility studies are reviewed. Finally, the reader is introduced to in vivo applications of diamonds. These include drug delivery, aiding radiology, labeling, and use in cosmetics. The field is critically reviewed and a perspective on future developments is provided.

1. Introduction

In the past years, nanodiamonds (NDs) have attracted increasing attention. No other element holds so many records in material properties. This unique combination of properties is responsible for the large variety of fascinating applications where nanodiamonds have been utilized. Differently sized and shaped diamonds having entirely different properties further increase the diversity of the topic. Different diamond materials have been utilized for a number of different applications. Surface chemistry, forms, and sizes of nanodiamonds define the applications they are useful for. In physics, electronic properties and their ability to host stable fluorescent defects which can be used as spin qubits are valued. These defects can also be used as stable light sources. Since they change their optical properties depending on their magnetic surrounding, they are promising nanoscale quantum sensors. They can be used to detect, for instance, magnetic or electric fields, temperature, pressure, or strain. The same defects are also very attractive for labeling since they do not bleach and are visible well in many different imaging methods. Chemists or biologists on the other hand value the small size (around 5 nm) of detonation diamonds, because it offers a relatively large surface to attach molecules. Additionally, they are inert but still can be functionalized on the surface. This qualifies them as excellent vehicles for drug delivery. Several different aspects of nanodiamonds and their applications have already been reviewed: magnetometry, surface chemistry, the physics of defects, drug delivery, biomedical applications, regenerative medicine, or a combination of several applications. We would like to provide a review article on in vivo applications. To our opinion, this topic is absolutely essential for any potential medical applications. This has not been reviewed before, despite the relevance and although there is already a substantial body of literature about this topic. We will first review what diamond materials are available and how they differ and summarize in which organisms diamonds have already been used. Then, we will discuss biocompatibility including survival and also more subtle non-fatal changes. Next, we will add a discussion on drug delivery and labeling, which are the two most wide-spread applications. Finally, we will also discuss newer or less common applications including use in cosmetics, implants, magnetic resonance measurements, or studying transport and motion. During the entire discussion, we will carefully differentiate between different diamond materials. This is often neglected in the literature and can lead to major confusions and/or conflicting results.

2. Diamond Starting Materials

There are several ways to produce nanodiamonds. Two of them have already been used in vivo. Depending on the method, the resulting material has fundamentally different properties. Thus, it is very crucial to specify exactly which material is used. In this section, we will describe how the materials are made and what the difference between them is.

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2.1. Detonation Nanodiamonds (DNDs)

The oldest method is to induce a controlled explosion of certain carbon containing compounds (typically TNT-like explosives). As a result, one obtains very small particles with a narrow size distribution and a size around 5 nm.[22] They are round or oval in shape and typically contain a high number of impurities and defects.[23] Also, they usually have sp² carbon (graphitic carbon) on the surface. Their small and reproducible size as well as their large relative surface area are most valued for drug delivery applications. These properties allow the attachment of large quantities of drugs and lead to reproducible particle sizes. Due to their small size and high amount of impurities, DNDs are less likely to host stable fluorescent defects. So, they are usually not the material of choice for labeling or sensing applications. Interestingly, Reineck et al. recently achieved to have stable defects in DNDs, so they might become more attractive for these applications too.[24]

2.2. Diamonds from Grinding

Very different particles are obtained by grinding from larger (typically high pressure high temperature (HPHT)) diamonds. They are more pure and contain fewer defects than DNDs. They have a flake-like shape[25] and are available in many different sizes. They usually have a very broad size distribution, but can also be size selected. An elegant way to do this is centrifuging.[26] Particles above a certain size will sediment and the supernatant then only contains particles below that size. The particle sizes can be adjusted by using different centrifugation speeds. These particles can naturally host stable defects.

2.3. Other Materials

Apart from these two materials, which are widely used, there are also new developments of materials, which have not yet been used in vivo but might play a role in the future. One option is to use a predefined adamantine (small molecule with a diamond-like position of molecules), which already has the atoms required for defects, and then grow a diamond around.

Finally, diamond nanoparticles can be also produced by microfabricating bulk diamond.[27] This approach allows using very pure starting material and offers some control over the shape of particles. Both of these methods so far suffer from low yields but promise superior defect properties for sensing or labeling.

3. Surface Chemistry

Due to their high surface to volume ratio, nanomaterial properties are largely defined by their surface chemistry. The smaller the particles, the higher the percentage of atoms that are actually on the surface. This is, of course, also true for nanodiamonds. “Naked” nanodiamonds are usually oxygen terminated as treatment with oxidizing acids or heating in air is an efficient way to remove non-diamond materials or impurities from the surface. These oxygen-terminated diamonds have a rich variety of oxygen containing groups on the surface, including esters, carboxylic acids, alcohol groups, or acid anhydrides.

Depending on the application, this surface termination has been altered or different molecules have been attached. An overview of the modifications that have been used and for what reason is given in Table 1. As surface chemistry of diamonds has already been extensively reviewed elsewhere,[10,15,28] we will here only briefly mention the points that are specific for in vivo experiments.

There are several reasons to alter the surface chemistry. One goal is to make the surface more uniform. This is often the first step to further attach other molecules as drug molecules, dyes, or molecules which provide selectivity (as antibodies). Having a uniform particle surface increases the number of useful binding sites and reproducibility. In applications where diamond defects are utilized, surface termination is crucial to preserve fluorescence and to stabilize defects (or a desired charge state of defects) and to avoid surface impurities.

There are also several reasons that are specific for biological applications. Preventing aggregation is important as naked nanodiamonds would not be colloidal stable in body fluids or cell media (due to the salt and protein content). Furthermore, the particles should be biocompatible and not interfere with

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Romana Schirhagl holds a PhD in chemistry, which she obtained in 2009 from the Vienna University and she has an additional education in biology. After two postdocs at the Stanford University and the ETH Zurich, she started her own group at the Groningen University. The aim of the group is applying diamond magnetometry to biomedical applications. Most recently, she was awarded with a prestigious ERC starting grant.
the biological function. Another concern is to avoid protein adsorption.

In addition to these requirements, there are also several points that are of importance for in vivo experiments. In animal experiments, coatings can be useful to avoid immune reactions toward the particles.\cite{29} It might also be important that the particle remains in the organism long enough (this plays a role in drug delivery).\cite{30} Furthermore, it is important that the particles can be cleared from the system and do not accumulate to toxic levels in an organ (for example, the liver).\cite{49} Unlike in cell experiments, the cells where the diamond should end up, as, for example, a cancer, has to compete with many other cells in the body. Thus, it is also important to avoid diamond accumulation in unwanted cells or organs.\cite{58}

4. Model Organisms

To perform the first in vivo experiments with nanodiamonds, different model organisms were chosen. An overview of the organisms that were utilized is given in Figure 1. Generally, commonly used model organisms were used, which represent different groups.

5. Biocompatibility

The first step toward using a material in in vivo experiments is to test its biocompatibility. This has been tested in detail in different cytotoxicity studies. Here, it is important to differentiate between the detonation nanodiamonds and the HPHT diamonds. For the HPHT diamonds, consistently, low or no effects on viability have been found.\cite{31–33} Also nonfatal influences have been considered and are generally considered low on different cell types.\cite{34} For the detonation nanodiamonds, the biocompatibility is overall a bit worse than for HPHT and different results are found for different surface chemistries.\cite{35–38} Here, we will focus on the in vivo studies. Initially, in vivo experiments were mainly performed in aquatic (fish and amphibian) species and more recently, also some safety assessment testing has been performed in mammals. For toxicity studies as well as for the drug delivery studies in the following section, it is of importance how the diamonds have been administered. Figure 2 gives examples for different routes that have been utilized to administer diamond nanoparticles or products made from them. Methods of ND administration to animals include subcutaneous or intravenous injections and intracheal instillation (= inhaling of particles by the animals), of which the latter is commonly

<table>
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<th>Surface termination</th>
<th>Purpose</th>
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<td>Drug delivery (see Section 6.1)</td>
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<tr>
<td>Bare (oxygen terminated) coated with drug</td>
<td>Simplicity</td>
<td>Drugs adsorbed and attached via oxygen containing groups</td>
<td>[56,60]</td>
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<td>–OH (then attach NH₄ containing groups and covalently attach drugs)</td>
<td>Increase uniformity, covalent attachment is necessary if the drug does not adsorb to ND</td>
<td>Tumor growth could be inhibited</td>
<td>[38]</td>
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<tr>
<td>Coated with targeting molecules (as TAT)</td>
<td>Increase uptake by cancer cells</td>
<td>Higher amount of ND/drug reaches the cancer</td>
<td>[58]</td>
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<td>Coating with PEG + drug</td>
<td>Prevent adhesion of proteins and immune responses</td>
<td>Prolonged circulation time, enhanced accumulation in tumor-metastasized lung</td>
<td>[59]</td>
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<tr>
<td>Improving implant properties (see Section 6.2)</td>
<td>Increase solubility in implant material</td>
<td>Nanodiamonds are well dispersed in the implant material and improve mechanical properties</td>
<td>[86]</td>
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| Labeling (see Section 6.3) | | | |
| Folic acid and dye linked via NHS linker* | Attach a molecule for targeting and a labeling molecule | Labeling of tumors in mice achieved | [95] |
| Bare (oxygen terminated) coated with molecule of interest | Labeling molecules | Transport of the labeling molecules of interest could be observed | [140] |
| Oxygen terminated coated with complexes of conventional MRI labels | Provide T1 contrast | The diamond-based contrast agent is well visible in MRI | [113,120] |

| Optical magnetic sensing (see Section 6.4) | | | |
| Bare (oxygen terminated) coated with drug | Stabilize defects | Proof of principle that contrast can be improved in vivo using the magnetic properties of ND | [122] |
| Biotinylated lipid-coated ND | Lipid contains labels for growth factors in cancer cells | Particles were accumulated in tumors | [97] |

| Use in cosmetics and sunscreen (see Section 6.5) | | | |
| Bare (oxygen terminated) | Simplicity | Protection from UV light by diamonds added to sunscreen | [125] |

| Studying transport and motion (see Section 6.6) | | | |
| Protein coated | Prevent aggregation | Motion of different parts of the embryo during embryogenesis was studied | [127] |

Table 1. Examples of surface modifications and their purpose for different applications. *An NHS linker is a common molecule which is used to attach different molecules which contain NH₂ groups.
used to investigate respiration toxicity of nanoparticles. The effect of the diamond particles on the animals was tested in several different ways, which are described below. An overview over different methods is given in Figure 3.

5.1. Survival Rates

No differences were found on the life span of worms exposed to fluorescent nanodiamonds (FNDs). Using a more sensitive model for biological toxicity, frog embryos, Marcon et al. found decreased survival rates of embryos in a certain stage of development after injection of 2 mg mL$^{-1}$ functionalized NDs in 2-cell embryos. After 14 d, 100% mortality was found in bivalves (mussels) that were kept in tanks with ND solutions of 10 µg mL$^{-1}$. This could partly be explained by the fact that bivalves are filter feeders and take up their food by filtering the water, which could result in high levels of ND uptake. In the other animal studies that were performed, no mortality rates have been measured as animals were sacrificed for histological analyses. Such histological analyses are performed to determine where in the animal diamond particles end up.

5.2. Morphological Effects

Embryonic development is a sensitive test for biological activity, as during the early stages of embryogenesis, the key aspects of cellular behavior, such as migration and proliferation, are recapitulated. No lethal toxic effects were observed after microinjection of nanodiamonds in embryonic clawed frogs, but sublethal malformations were found depending on the functionalization of the diamonds. Marcon et al. showed a higher induction of malformations after microinjection with ND$_{\text{CO}_2\text{H}}$. Microinjection with ND$_{\text{OH}}$ or ND$_{\text{NH}_2}$ did not result in different number of malformations as compared with water-microinjected embryos. In another study, using an embryonic zebrafish model, a concentration-dependent effect of NDs on malformations was found. The number of malformations showed an increase with increasing concentrations of NDs.

Histological evaluation of organ tissues after ND treatment showed mostly minor changes in heart and liver tissues. Administration of 1 or 2 mg NDs per week to rats resulted in relatively mild changes in liver tissue, potentially reversible. Substantial abnormalities were found in monkey heart and liver tissues on exposure to high ND doses of 25 mg kg$^{-1}$, but these abnormalities were considered less severe at standard doses of 15 mg kg$^{-1}$. In the lung, no ND-induced pathologies were detected in rats and monkeys, but an inflammatory response was observed after histological evaluation of murine lung tissue. Moore et al. did not observe any morphological changes in kidney and spleen tissue samples of rats and monkeys after ND administration of different doses.

5.3. Metabolic Responses

Although Moore et al. showed some histological alterations, no organ dysfunction was found by detecting several biochemical parameters in the blood serum of rats and monkeys. Puzyr et al. found some changes...
in blood biochemical parameters, indicating that liver function and lipid metabolism are influenced, although no signs of cellular destruction were observed.\textsuperscript{43} Likewise, Zhang et al. observed increased dose-dependent lipid peroxidation in alveolar compartments in mice.\textsuperscript{39}

Additionally, by evaluating the biochemical parameters in the blood, they had also found an indication of adverse effect on both the liver and kidney functions. To investigate the immune response of mice to ND injection into the blood stream, the level of an inflammatory protein (TNF-\(\alpha\)) in murine blood was analyzed. TNF-\(\alpha\) production was not increased in response to ND injection, indicating the lack of an ND-induced immune response (Tsai et al. 2016).\textsuperscript{47}

The expression levels of several role players in the oxidative stress response have also been evaluated, such as glutathione S-transferase (GST), catalase, glutathione (GSH), and malondialdehyde (MDA). While MDA is a marker for oxidative stress itself, the others are important enzymes, which are involved in oxidative stress and in protecting the cells from oxidative damage. Increased GST levels have been observed after 7 d of ND exposure in an aquatic species, freshwater bivalves (mussels). Increased catalase activities were detected after 14 d of exposure to NDs.\textsuperscript{42} Remarkably, this increase in catalase activity was not detected at the highest test concentration (10 \(\mu\)g mL\(^{-1}\)), indicating a role for catalase in protecting the cells only at low ND concentrations. Examination of the levels of oxidative stress parameters of murine lungs exposed to NDs did not reveal any significant changes in GSH and MDA levels in homogenized lung tissue samples;\textsuperscript{46} but did show a dose-dependent increase of MDA levels in samples of alveolar content.\textsuperscript{39} Analysis of reactive oxygen species (ROS) levels directly, did not show an induction of ROS production in worms.\textsuperscript{40}

5.4. Biodistribution

NDs injected into the blood stream of rats were shown to attach to red blood cell membranes, after 30 min of circulation in the blood system. This reveals that NDs can remain in the blood circulation for several cycles of the blood circulation without being excreted.\textsuperscript{47} Two hours after administration, NDs showed to accumulate mostly in liver and lung tissues.\textsuperscript{38,48,49} Since the liver is the organ, which is responsible for clearing particles from the blood accumulation, there is an expected finding. Additionally, Zhang et al. observed an inflammatory response in the lung, possibly as a result of the high, dose-dependent retention of NDs in the lung.\textsuperscript{39} They propose that this dose-dependent pulmonary toxicity could be mediated by the increased oxidative stress levels. In the long term, Yuan et al. showed a retention of NDs in murine lung and liver as 28 d later, they were still mostly accumulated in lung and liver.\textsuperscript{49} More specifically, they were observed to be accumulating in macrophages in the liver, suggesting that the NDs were captured by the reticuloendothelial (macrophage immune) system. Similarly, NDs were found to accumulate in macrophages in the respiratory tract up to 28 d after administration via the respiratory tract in mice.\textsuperscript{46} and in macrophages in the deepest surface of the abdominal wall, the peritoneum, after injection into this peritoneum in rats.\textsuperscript{50}

Not much is known on the excretion of NDs from a body. In rats, excretion of NDs was found to take place via the urinary tract,\textsuperscript{46} while the excretion in urine and feces was barely detectable in mice.\textsuperscript{49}

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**Figure 3.** Visualization of the aspects of biocompatibility that have been measured for using nanodiamonds in in vivo model systems, clockwise: survival rates, morphological effects, biodistribution, and metabolic responses. Survival rates of animals after injection with NDs\textsuperscript{38} or after exposure to NDs for certain time periods,\textsuperscript{37,39} e.g., 100% mortality was found in mussels after 14 d of ND exposure.\textsuperscript{39} Morphological effects after microinjection of nanodiamonds in early stage embryos, e.g., malformation in different embryonic developmental stages in zebrafish\textsuperscript{49} or clawed frogs.\textsuperscript{39} Biodistribution analysis of NDs after injection into the blood stream showed accumulation in the lung and the liver in both mice and rats.\textsuperscript{36,44–46} Metabolic responses are monitored by measuring the biochemical parameters, for example, in the blood circulation system of monkeys,\textsuperscript{41} rabbit,\textsuperscript{42} mice,\textsuperscript{36,43,44} giving an indication of organ (dys)function.
To conclude, it can be said that these findings collectively indicate that NDs are well-tolerated (see Table 2 for an overview of the findings). The in vivo studies combined with the absence of severe cytotoxic effects confirm the potential of nanodiamonds for medicine-related applications. Before clinical translation of ND-based therapeutic agents, comprehensive pharmacokinetic analyses are required.

6. Diamonds for In Vivo Applications

6.1. Application of Nanodiamonds in Drug Delivery

Over the past decade, increasing attention has been focused on drug delivery systems, because therapeutic agents can be efficiently coupled to them and applied to treatment of various...
diseases. The controlled delivery and release of therapeutic agents are very important. Drug delivery systems can provide different advantages, i.e., high local concentration of drug, selective targeting, stability of drugs in physiological environments, and lower side effects of therapeutic agents.\[51\] By targeted drug delivery, it is possible to tailor the dosage of different therapeutics to achieve the therapeutic action over a longer time with lower side effects. NDs and in particular DNDs are attractive for this application as they are small and have a narrow size distribution. In addition, although they are very inert, they offer a rich surface chemistry that can be altered. The charge properties on ND facets enable them to bind with water and acquire good aqueous dispersibility.\[52\] These properties make NDs serve as a good translational platform for disease treatment. Last but not the least, they are also very biocompatible.

6.1.1. Mechanisms of Drug Attachment to NDs

The simple mechanism of drug deposition onto ND surface is another advantage that makes NDs convenient as drug delivery devices. The drug can be attached either covalently or noncovalently. To achieve noncovalent attachment, drug molecules are mainly bound to the NDs via electrostatic interaction and hydrogen bonding between the drug molecules and the hydroxyl or carboxyl groups on the surface of the NDs. This adsorption mechanism is then followed by a desorption process for the targeted delivery and triggered drug release, mostly by altering the environment condition from basic to acidic. The large ratio of surface area to volume and high adsorption capacities of NDs play a critical role in the noncovalent interaction between the NDs and the different biological molecules. The noncovalent binding is simple, broadly applicable, and the structure of the drug and its bioactivity is exposed to minimal change. Also, the triggered drug release occurs easy and fast in response to environmental stimuli.

In addition to noncovalent interactions, loading of drug molecules onto NDs by covalent binding has also been reported. The covalent linkage of paclitaxel to NDs (DNDs, 3–5 nm in size) through a succession of chemical modifications has been reported by Liu and co-workers.\[57\] In an in vivo treatment, ND–paclitaxel markedly blocked the tumor growth and formation of lung cancer cells in xenograft SCID mice. But, in comparison to free drug or drug–carrier complex, covalent drug–carrier conjugate had lower anticancer activities of chemotherapeutics. To overcome this, NDs can be incorporated into the cell penetrating peptide trans-activator of transcription (TAT). This peptide is taken up by cancer cells and thus more of the drug reaches the cancer.

To have a successful drug delivery platform, specific and sustained drug release at the target site together with minimum loss of its volume and activity during the blood circulation is required. This strategy increases the concentration of drug in target cells and reduces the dose limiting toxicities.\[53\] Premature release of the drug will induce toxicity in the blood circulatory system, causing damage to normal cells and tissues. Figure 4 gives an overview of the strategies that have already been tested in vivo.

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**Figure 4.** Examples of different drug delivery strategies that have been tested in vivo. The strategies include the delivery of genetic material (1),\[70\] (2),\[71\] embedding the particles into a polymer film or scaffold (3),\[79\] (4)\[81\] as well as attachment of a number of different cancer drugs or combinations of cancer drugs and molecules that promote accumulation in cancer cells (5),\[51\] (6),\[51\] (7),\[88\] (8),\[87\] (9).\[78\]
6.1.2. Cancer Therapy

Different studies have shown that NDs demonstrate significant potential as gene/drug delivery platforms for cancer therapy. They are used as drug delivery devices either in hydrogel form or as ND films. Hydrogels are clusters of NDs in aqueous solution with size 10–100 nm. Drugs can be mainly attached onto ND clusters in a noncovalent manner.

In 1995, Kossovsky and coworkers used ND coated with cellobiose, a disaccharide, for immobilization of mussel adhesive protein (MAP). The diamond–cellobiose–MAP complex was injected into New Zealand white rabbits, and their specificity against MAP was measured. The rabbits showed a strong and specific antibody response due to antigen delivery. Moreover, the conformational stabilization of the protein when immobilized on ND surface resulted in better antibody binding. The complexes of ND–drug had no negative effect on the number of white blood cells. This was promising for cancer treatment.

A number of pioneering studies of drug delivery systems based on NDs have been conducted by Ho and coworkers. They demonstrated that ND clusters are capable of complexing with poorly water-soluble drugs to enhance their dispersive properties in water. So, a number of water-insoluble anticancer drugs, such as doxorubicin (DOX) hydrochloride, a protein kinase inhibitor purvalanol A (a medicament for liver cancer), 4-hydroxytamoxifen (a drug for the treatment of breast cancer), and anti-inflammatory drug dexamethasone were adsorbed onto ND hydrogel through noncovalent interaction to form a ND–drug complex. The passively targeted delivery of these drugs by nanodiamonds has been shown to increase therapeutic efficacy against multiple models of cancer. These works showed that NDs increased the solubility of the absorbed molecules, even if they are highly hydrophobic.

DOX is one of the most common anticancer drugs, which induces cell apoptosis and is widely used in cancer therapy. However, because of dose-related toxic side effects such as cumulative cardiotoxicity and myelosuppression, its clinical application is limited. Using DOX with nanodiamonds has been extensively studied in different approaches. Ho and coworkers absorbed DOX hydrochloride onto NDs (2–8 or 45 nm in size) through electrostatic interactions between the protonated amines on the DOX molecules and the deprotonated carboxylic acid groups on the NDs. DOX and NDs interact only weakly under ambient conditions due to low aqueous solubility, as many other drugs with therapeutic ability do. This leads to limitations of their potential application because loading capacity is very important in drug delivery systems. They found that introducing a small amount of some inorganic molecules such as NaCl to the drug delivery system could promote the drug loading onto NDs by increasing drug solubility. The addition of 10 mg mL\(^{-1}\) NaCl increased the adsorption of DOX on NDs from 5 wt% to over 10 wt%. The reversible release of DOX was also possible by regulating the Cl\(^{-}\) concentration. The activity of ND–DOX complex was investigated in mouse models of liver and mammary cancer.

Because of the surface modification capability of NDs, different approaches have been invented to improve the applicability and the function of drug delivery platforms based on NDs. ND–DOX complex when further modified with 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG 2K), abbreviated as DNX, can inhibit the lung metastasis starting from 4T1 breast cancer cells in mice. This modification helps enhancing the transmembrane ability of the ND–DOX complex to enter into the nucleus and inhibit the synthesis of nucleic acid through intercalation with DNA as well as increase dispersibility and prolong circulation time. The in vivo studies done by injecting the DNX formulation intravenously into nude mice, bearing lung metastasized breast tumor cells, showed good drug accumulation in lung and markedly inhibited the lung metastasis of breast cancer.

FNDs with sizes greater than 100 nm (i.e., 140 nm) were also modified with DOX for in vitro and in vivo studies. The blood vessels in tumor tissue can retain particles of this size. Confocal fluorescent microscopy on Hela cells showed that the free DOX could enter the nucleus, but FND–DOX were distributed in the cytoplasm and could detach DOX more slowly to migrate to the nucleus. For in vivo studies, bare ND (mean diameter 140 nm) and an ND–DOX complex (mean diameter 165 nm) were administrated by intraperitoneal injection in a tumor bearing mouse model. In vivo, tumor-bearing mice treated with ND–DOX survived four times longer than mice treated with free DOX.

Although DOX has already been a cornerstone of chemotherapy for decades, it has not been considered for clinical chemotherapeutic treatment of malignant brain tumors yet due to poor penetration across the blood–brain barrier. To overcome this poor penetration, Xi et al. reported convection enhanced delivery (CED) of ND–DOX complex as a new therapeutic strategy in glioma cell lines and in a rodent brain tumor model. CED is a new therapeutic strategy to inject ND–DOX directly into rodent brain tumor model. Administering ND–DOX via CED resulted in increased apoptosis in glioma cell lines in in vitro studies and in improved drug distribution and retention in brain tissue of treated rats in vivo. ND–DOX delivered via CED increased DOX uptake and retention in rodent brain parenchyma, and was significantly more efficient at killing tumor cells than uncomplexed DOX. Accordingly, ND–DOX has been introduced as a promising approach for brain tumor treatment. A slightly more complex but targeted approach is to add a targeting molecule in addition to the anticancer drug. Ryu et al. used folic acid for this purpose. Many cancer cells, including oral cancer (KB) cells that were used in this study, have receptors for folic acid and thus, this molecule helped to achieve targeting. The authors tested their particles in mice that were previously injected with so-called KB cells, which lead to tumor formation. As expected, they found that the diamond particles that contained both folic acid as well as doxorubicin were the most potent in inhibiting tumor growth. An alternative for folic acid that has been utilized is transferrin. It accumulates in certain tumors since they actively express receptors for this molecule on the cell membrane.

Another approach to combine doxorubicin with an additional molecule was shown by Li et al. They attached doxorubicin onto diamond particles that were coated with polyethylene glycol (PEG). PEG is well known for its excellent properties when it comes to suppressing immune responses. Similar to
the previous studies, they observed inhibition of tumor growth. Another effective ND-based drug delivery system was shown by Lin et al., targeting colorectal cancer in mice. The code- delivery of paclitaxel (PTX), a microtubule inhibitor, and cetux- imab (Cet), inhibitor of an important growth factor, was found to enhance tumor inhibition in mice with induced colorectal cancer. This is a relevant finding, since multidrug delivery is often prescribed in cancer patients. PTX and Cet were covalently linked to NDs, prior to administration in nude mice. Although this study showed an enhanced effect on tumor inhibition, there was no specific information on the route of administration and circulation stability of this multidrug delivery complex.

Deng et al. produced theranostic diamond-based particles, which have both a therapeutic function to kill/suppress the tumor, as well as a visualizing function to provide a platform for imaging. To achieve this, they attached microRNA, cyanine, and protamine sulfate to DND clusters. MicroRNA is the active compound in the complex. They are often abnormally expressed in cancer cells and act as oncogenes or tumor suppressor genes. The particles were tested on mice containing esophageal tumors. Cyanine is a dye molecule that emits in the near-infrared and allows to visualize the particles and the tumor. The function of protamine sulfate is to bind and protect the other two substances at physiological pH (7.4). When the pH drops (as it is the case when the particle is taken up by a tumor cell), the particle releases its cargo. The authors were able to accumulate their nanoparticles in the tumor, where it both allowed imaging as well as inhibited tumor growth.

As an alternative to microRNA, also small interfering RNA (siRNA) has been attached to diamond and successfully delivered into tumors. These siRNAs are potentially very powerful tools since they can silence genes inside the cancer cells. After administration of siRNA–ND complexes, the authors demonstrated a downregulation of survivin protein expression (a protein which is overexpressed in tumors) that resulted in apoptosis of tumor cells.

6.1.3. Drug Delivery Systems against Chemoresistance of Cancer

Chemoresistance (resistance to chemotherapy) is the major reason for treatment failure in cancer therapy, particularly in metastatic cancers. Even chemosensitive cancers after some treatment acquire chemoresistance and even cross-resistance against other chemotherapeutics of standard chemotherapy. This is called multidrug resistance. After exposure to only one single drug, multidrug resistance to a wide variety of therapeutics can occur. One cause for this drug resistance is the overexpression of drug export pumps such as P-glycoprotein (ABCB1 protein), ABCG2 protein, or ABCC1 protein. Adenosine triphosphate binding cassette (ABC) transporter protein can recognize and efflux drug molecules from tumor cells against the concentration gradient. This leads to reductions in intracellular drug concentration and subsequent progression of drug insensitivity. For example, DOX as a standard chemotherapeutic can be effluxed from tumor cells by a wide range of drug transporter proteins, including MDR1, ABCG2, and MRP1. ABC transporters are capable of effluxing several classes of drugs, including the anthracyclines such as doxorubicin and epirubicin, as well as the antitubercenoides such as mitoxantrone. To overcome multidrug resistance, ABC transporter must be inhibited. Nanodiamonds are reported to act as a good platform to compensate efflux pumps in the treatment of chemoresistance cancer cells. The ND–DOX complex used in the work of Wang et al. demonstrated the ability to overcome drug efflux in vivo from tumor cells and increase apoptosis in liver tumor and mammary carcinoma models compared to standard DOX treatment. This is due to the fact that the drug–ND complex is very different from free drug molecules in terms of size and structure and does not fit the requirements of ABC transporter substrate. Hence, the ND–drug complexes cannot be recognized as ABC transporter substrate and cannot be effluxed out of tumor cells. The longer period stay of drug within the cell leads to slow and sustainable release.

Another study that shows that ND–drug complexes can help prevent drug efflux and chemoresistance is reported by Wang et al. in 2014. Epirubicin drug is another member of the anthracyclines family, like DOX, but of lower cardiotoxicity than DOX. It inhibits DNA and RNA synthesis and DNA replication and it can be recognized and effluxed by multiple ABC transporters. In the work of Wang et al, the epirubicin–nanodiamond complexes (EPND) were formed simply by physical adsorption of the drug onto the ND surface. The resulting EPND complex was successful in killing chemoresistance hepatic cancer cells. The association of epirubicin to NDs prevents efflux of the drug by ABC transporters. Therefore, the drug retains longer in the cell and, as compared to unmodified epirubicin, exhibited higher efficacy in killing both the normal cancer cells and the cancer stem cells in vitro (LT2-MYC cells) and in vivo. It is worth mentioning that, this is a specific function of ND-mediated drug delivery systems because epirubicin delivery by the highly used nanomaterial vesicles was not successful in drug retention enhancement within the cell compared with free epirubicin or EPND.

In vivo experiments showed that although both epirubicin and EPND cause a strong apoptotic response, EPND-caused apoptosis was limited to tumor tissue and did not occur in adjacent normal hepatic tissue. This suggests that NDs can serve as an effective and safe drug delivery platform to target and eliminate chemoresistant cancer cells. However, as a side effect, noncancer stem cells are targeted as well. The acidic environment inside cells and the intracellular presence of charged protein were the stimuli to detach epirubicin from nanodiamonds to detach epirubicin in the right place. Another approach to deal with chemoresistance can be found in multidrug delivery systems, for example, the earlier mentioned ND–PTX–Cet complex reported by Lin et al. They suggest that their multidrug delivery complex, ND–PTX–Cet, that increased cell death through both mitotic catastrophe and apoptosis, could be used to deal with chemoresistance tumors as well. By improving both the drug retention and the treatment efficacy, they argue that their multidrug delivery complex can help to overcome drug-resistant forms in human cancer patients.

6.1.4. Other Drug Delivery Applications than Cancer

Ryu et al. demonstrated targeting in mice into the bone. In order to achieve targeting, the authors coated nanodiamonds
with alendronate. This is a small molecule with a high affinity to hydroxyapatite and thus, bone material. The authors could show that the coated diamond particles are preferably ingested by bone cells and accumulate in bone tissue after injection into the tail vein. The authors suggest to use this method for delivering osteoporosis drugs or alendronate itself.

So far, nanodiamonds are mainly investigated for the delivery and sustained release of anticancer chemotherapeutics.\cite{38,76} However, many different molecules and drugs such as cytochrome c\cite{77}, DNA,\cite{78,79} antibodies, fully functional proteins,\cite{80} and various protein antigens\cite{54} have also been conjugated with NDs. These offer interesting drug delivery options in the future.

### 6.1.5. Applications of ND Film in Drug Delivery Systems

Another approach for drug delivery is to use a scaffold that delivers the drugs. Incorporation of drug-loaded nanodiamonds into such a scaffold was recently employed by Suliman et al. in mice.\cite{81,82} They used a copolymer containing poly l-lactide and ε-caprolactone as monomer units in which they incorporated the detonation nanodiamonds which were coated with a bone morphogenetic protein (BMP2). This protein promotes bone formation and also plays an ambivalent role in tumor formation. One aim of this study was to further investigate and improve this side effect. The diamond particle should prevent quick release bursts of the protein from the scaffold. When tumor formation was induced, these nDP-modified scaffolds reduced the size of tumors in vivo. The authors, however, recommend caution when using BMP-2, due to its poorly understood role in cancer formation.

Another study that is worth mentioning here was done by Lee et al.\cite{83} The authors implemented antibiotic-coated nanodiamonds into gutta-percha material which was used for root canal treatment. The antibiotics that were released from the material indeed fulfilled their purpose and inhibited bacterial growth. Strictly speaking however, this is not an in vivo experiment since the experiment was done on teeth that were extracted from patients. In a follow up study, they report administration of the thermoplastic biomaterial NDGP in humans.\cite{84} NDGP was used as a root canal filling material, to prevent both reinfection and bone loss as a result of root canal therapy. In a small interventional treatment study with one control and three NDGP-treated patients, they show effective lesion healing and no adverse events were observed. This indicates the potential therapeutic contribution of the NDGP biomaterial and provides information on the clinical tolerance of using DNDs for therapeutic applications.

### 6.1.6. Drawbacks and Current Bottlenecks

From the discussion in Section 3 about all the requirements that need to be fulfilled in drug delivery, it is clear that this is a very complex topic. As a result, although there are several groundbreaking results, it is not easy to translate this to other systems and often there is a lot of trial and error involved. Delivery to a different location for a different type of cancer can change the situation entirely. However, it has to be noted that this is a problem that is not specific to nanodiamonds, but affects the entire field. A diamond specific issue, which is currently addressed by the community, is the application of coatings in order to prevent aggregation.

### 6.2. Nanodiamonds for Improving Properties of Implants

In implants, nanodiamonds offer the advantage that they are very hard materials and thus improve mechanical properties of the composites. Similar to the previous applications, the ability to adjust the surface is also beneficial here. In order to promote bone formation, Zhang et al. incorporated nanodiamonds that were coated with phospholipids inside a poly(lactic-co-glycolic acid) matrix.\cite{85} The difference to the previous article (Suliman et al.) is that here, the phospholipids are not a drug compound but merely help to suspend nanodiamonds in the polymer material.\cite{83} The material was implanted in mice where the polymer was degraded (slower in presence of diamond) and is replaced by new bone material. When diamonds are present in the material, the mechanical properties of the material are more favorable for bone formation as well. The authors show acceptable immune responses toward the implanted material.

### 6.3. Labeling

Another important application of nanodiamonds is labeling. Here, the HPHT diamonds are more suitable than the detonation nanodiamonds due to their ability to host one or more defects. There are hundreds of diamond defects known today, covering the entire visible spectrum. The most prominent examples are the so-called nitrogen vacancy center and the silicon vacancy center, which both emit in the red. Over conventional dyes, diamond particles have the advantage that they do not bleach. Consequently, they are suitable for long-term measurements. The second advantage is that they are visible with several different imaging methods. They are visible in fluorescence microscopy where their emission in the far red (between 600 and 800 nm) is valued for in vivo studies due to the low background in this region. Nanodiamonds are also well visible in fluorescence lifetime imaging (FLIM) since their fluorescence lifetime (11.6 ns for bulk diamond\cite{86}) is considerably longer than that of typical biomolecules (1–4 ns\cite{87}). Furthermore, they are well visible in near-infrared imaging, photoacoustic imaging, magnetic resonance imaging, and cathodoluminescence imaging, where both Si-V (silicon vacancy centers)\cite{88} as well as neutrally charged N-V centers are visible.

Thus, nanodiamonds can be very useful labels when images from different methods should be correlated.\cite{80} Here, we focus on ND-based imaging which are performed in vivo.

#### 6.3.1. Near-Infrared Imaging

Near-infrared (NIR) fluorescence imaging is one of the most important burgeoning in vivo imaging modalities in...
fundamental research and clinical practice.\[^{[89,90]}\] NIR is the most valued in vivo imaging for its wide wavelength range (from 700 to 1700 nm) and the good tissue penetration. This allows taking measurements deep within a tissue. NIR imaging technique, for instance, can give optical resolution data in a tissue depth of $\approx 3$ cm.\[^{[91–93]}\] NDs (both the HPHT- and the detonation-based ones) were utilized in this field already in preclinical NIR imaging studies.\[^{[94]}\] NIR labeling is achieved by conjugation with NIR-emitting dyes either via covalent bonding (e.g., folic acid\[^{[95]}\] and gold\[^{[96]}\]) or via noncovalent bonding (e.g., 750 – XenoLights CF750,\[^{[97]}\] Cyanine 5\[^{[67]}\]) (see Figure 5). This was used, for instance, in cancer diagnosis, by using modified NIR probes attached to NDs. These labels were intravenously administered as NIR imaging contrast. Either laser or light-emitting diode (LED) was used for NIR excitation. Once the ND-NIR probe locates on the tumor, a sensitive detector collects the optical signals. Due to the appropriate size of NIR-NDs, they can significantly increase the retention time.

6.3.2. Photoacoustic Imaging

Photoacoustic imaging (PAI) is another type of photoductive bioimaging modality that is used in preclinical studies for diagnosis.\[^{[98]}\] PAI has even greater imaging depth ($\approx 8$ cm) and resolution ($\approx 100$ $\mu$m) than the previously mentioned method in 2D- or 3D-models.\[^{[99,100]}\] Similar to NIR imaging, the principle of PAI is based on NIR laser excitation. However, unlike NIR imaging, PAI detects ultrasound signals generated from photoacoustic effects (see Figure 6).\[^{[101]}\] DNDs are promising PAI contrast agents, not only due to their biocompatibility and the straightforward labeling process, but also due to their high optical absorbance.\[^{[102–104]}\] As shown in Figure 6, NDs absorb energy of nonionizing laser pluses and convert it into heat, resulting in thermoelastic expansion and ultrasonic waves. Meanwhile, the ultrasound signal is monitored via ultrasonic transducers.\[^{[105]}\]

6.3.3. Magnetic Resonance Imaging

3D magnetic resonance imaging (MRI) is one of the most widely used imaging modalities in clinical diagnosis as well as preclinical studies. The advantages of the technique are its noninvasiveness and the ability to differentiate between elements. Additionally, high spatial resolution of a few millimeters to a few micrometers can be achieved.\[^{[106–109]}\] MRI signals are caused by proton spinning induced by external magnetic field ($B_0$).\[^{[110,111]}\] In order to have more credible images, imaging contrast agents are applied.\[^{[112]}\] Two types of MRI contrast agents are existing, one is $T_1$ contrast which reduces spin–lattice relaxation ($T_1$) time and the other is $T_2$ contrast which reduces spin–spin relaxation ($T_2$) time.\[^{[112–113]}\] $T_1$ contrast agents have paramagnetic nature (e.g., Gd(III)\[^{[114]}\] and Mn(II)\[^{[115]}\]), while $T_2$ contrast agents are super-paramagnetic materials (e.g., iron oxide,\[^{[116]}\] iron–platinum\[^{[117]}\]). Recently, contrast agents which are visible in both the $T_1$- and $T_2$-weighted images are attracting lots of attentions.\[^{[118,119]}\] Figure 7 shows an overview of how nanodiamonds are visible in different modes of MRI. Meade and co-workers\[^{[120]}\] and Chow and co-workers\[^{[113]}\] found that NDs can be applied as a promising MRI contrast nanoparticle. Some of their results are shown in Figure 7. NDs are potentially useful as a label by themselves in $T_1$ imaging due to their paramagnetic lattice defects. However, they are also promising as carriers when traditional contrast agents (e.g., gadolinium-based complexes or manganese ions)\[^{[112]}\] are attached. In addition, the HPHT diamond can act as magnetic material due to ferromagnetic inclusions (e.g., iron, nickel, etc.) which can be included during the manufacturing process.\[^{[121]}\] Thus, NDs are potentially useful for both the $T_1$ and $T_2$ contrasts and might be attempted as a potential dual-mode MRI contrast agent.

\[\text{Figure 5. The mechanics of ND-based NIR fluorescence imaging. NIR emitting dyes (indicated by an orange star) are linked to the diamond covalently (1) or adsorbed nonspecifically (2). The composite material is injected into mice. Excitation is performed with a laser or LED in the NIR range and is read out optically.}\]

\[\text{Figure 6. The mechanics of ND-based photoacoustic imaging. Blue color indicates medical ultrasound gel which distributes between the skin and the detector for ultrasonic signal conduction.}\]
emit red photons when they are illuminated with green light.

As described before, N-V centers are paramagnetic defects in the diamond itself or by coating with conventional contrast agents. T2 contrast has been achieved via metallic impurities in the diamond material. A T1-weighted image which was taken from mice liver, a tumor indicated by the arrow. The images were taken using nanodiamonds as MRI contrast agents. (b,c) Reproduced with permission. Copyright 2017, Elsevier.

6.3.4. Drawbacks and Current Bottlenecks

Currently, the main drawback of nanodiamonds remains the relatively large size compared to conventional organic dyes. While a typical dye only consists of a few tens of atoms, nanodiamonds are considerably larger. Using smaller nanodiamonds comes at the expense of losing brightness. As a result, diamonds are only a good alternative when long-term labeling is necessary or when the structures, which should be labeled, are large and the size is less critical (for example, for labeling cells). In in vivo applications, this is even more critical when imaging should be done within a tissue and the brightness does not penetrate that deep. To circumvent this problem, using infrared instead of optical excitation might offer a solution as the background is lower and tissue penetration is better.

When using nanodiamonds as MRI contrast agents, metal ions as gadolinium or iron easily outperform nanodiamonds when it comes to the contrast they provide. However, they do offer advantages if images should be correlated with different imaging modalities since nanodiamonds are well visible with many techniques. Additionally, they can function as good carriers for the traditional contrast agents.

6.4. Optical Magnetic Sensing

Arguably, the most interesting property of nanodiamonds and their defects is that their optical properties change based on their magnetic surrounding. As described before, N-V centers emit red photons when they are illuminated with green light. However, apart from this optical transition, the N-V center also has magnetic states. Depending on which state the diamond defect was in at the time of illumination, it has a different brightness. Thus, if a transition from one state to the other is induced, this results in a drop in fluorescence. At which microwave energy (= position in the spectrum) these drops in fluorescence appear and the distance between these peaks, give a measure for different quantities as temperature or magnetic field.

This concept was applied for diamonds that were introduced into the intestine of Caenorhabditis elegans (C. elegans). The authors used the fact that nanodiamonds change their fluorescence at a certain microwave energy to eliminate the background signals. Only the signal which changed upon microwave radiation was identified as the diamond signal. Light that was constant when irradiated, was identified as the background fluorescence. This is a property that is very specific for diamond defects. Whether there are other materials that can change their fluorescence in a similar way during microwave excitation and that are biocompatible, remains to be seen.

6.4.1. Drawbacks and Current Bottlenecks

While this method could potentially offer the possibility to sense all kinds of interesting molecules, in practice this is still largely unexplored. Currently, the main bottleneck is that nanodiamonds have relatively high amounts of impurities, which decreases the sensitivity compared to bulk diamonds. A solution would be to produce better quality diamonds. However, these are not yet easily available. Additionally, problems concerning brightness which were discussed earlier apply here as well.

6.5. Use in Cosmetics and Sunscreen

Another application where nanodiamonds are in use is cosmetics. There are several reasons why diamonds are added to cosmetic formulations. Adding nanoparticles in general offers the possibility for biomolecules to adhere and thus, in some cases, increased bioactivity can be achieved. Additionally, there are also some arguments that are specific for diamond. The first is its exquisite reputation. However, there are also other benefits from adding diamonds to cosmetics. Antioxidative behavior has been found in nanodiamonds. Nanodiamonds can also absorb light. Both the properties can add to a protective effect in sunscreen or other products that protect from light. This concept has been patented in 2005. The UV protection of the skin by nanodiamonds was also tested on cells and on mice. The authors found that 2 mg cm⁻² of NDs efficiently...
reduced over 95% of UVB radiation. This value is comparable with the values for two commonly used nanoparticles (titanium oxide 99%, zinc oxide 90%) that are added to sunscreen. However, less oxidative stress was observed with the nanodiamond. The study also compared 5 and 100 nm diamonds. 100 nm NDs performed better in terms of biocompatibility with cells and were thus further used in the mouse model. The penetration of the skin was also investigated and 100 nm diamonds were only found in the uppermost skin layer, even skin that was highly sunburned prior to administration. Due to unclear long-term effects, the authors suggest to avoid accumulation and retention in the body.

6.6. Studying Transport and Motion

The striking advantage diamonds brings for this application is its stable fluorescence (compared to conventional dyes which usually undergo bleaching) which allows long-term studies.

One long-term application is to study protein transport. This was first investigated in vivo with nanodiamonds by Kuo et al. They used the stable emission from fluorescent (HPHT) nanodiamonds to study protein transport.[140] To visualize the transport of these proteins from the intestines, they first coated diamonds with the proteins. Then, they were injected into intestinal cells. The authors observed secretion to the pseudocoelomic space followed by endocytosis into oocytes (egg cells). This is exactly the fate one would expect from the lipoprotein alone. The diamonds were also accumulated in the embryos that grew from these eggs. Figure 8a,b shows some of the results of their study. The authors noted that the protein transport was not disturbed by the presence of the diamond. However, for many protein transport studies, the size of the diamond might become problematic. Especially, when proteins pass membranes and should be unfolded, refolded, and modified in the process, large diamond particles will likely perturb the process. Using smaller diamonds could improve this problem, but smaller diamonds (containing less defects) might not be bright enough for FLIM. Another concern is that the adsorption of protein was done nonspecifically. Such adsorption is fast and simple and has been proven successful for a number of proteins. However, also some proteins in cells or cell medium tend to stick to the diamond surface[126] and might compete for the diamond surface with the proteins that were initially attached. While this was not a problem in the presented study, it could be a problem for proteins that bind less well to the diamond surface.

Another application is to monitor movement of entire cells or parts of an embryo. This was tested in Drosophila melanogaster, a fruit fly and also one of the most important model organisms in genetics and development biology.[127] In their study, nanodiamonds were injected into the Drosophila embryos. During the development, the authors recorded the movements that diamond particles experienced due to the development of the embryo. In their proof of principle study, the authors were able to observe movement from individual particles and differentiate between a directed movement and the free diffusion through the embryo.

6.6.1. Drawbacks and Current Bottlenecks

For this application, the drawbacks are quite similar to the ones that have been discussed in Section 6.3.4 about labeling. An additional issue here is that the motion of small molecules (as, for instance, proteins) might be influenced if there is a comparably large diamond particle that has to be transported in addition. The only way to avoid this is to compare to proper controls. There is also a lot of large enough systems in which it is interesting to study motions and the size of nanodiamonds becomes negligible (an example is to study the motion of entire cells or organelles).

7. Conclusion

Comprehensive knowledge about ND biocompatibility and toxicity to in vivo model systems is already available. Among applications that make use of the small size and inertness,
drug delivery has already been tested the most in vivo. However, no diamond-based drug delivery materials have so far been used on humans. And, despite the promising results in animal experiments, there are no clinical trials yet. However, for several diamond-based drug delivery systems, clinical trials are already the logical next step. A promising new application is, for instance, the use of nanodiamonds to assist radiotherapy that has been shown in vitro but not yet in vivo.\[128\]

The applications that utilize the unique optical properties of nanodiamonds are even more in their infancy. The unprecedented stability of defects paired with biocompatibility are ideal for long-term observations. However, the low brightness or the need to go to larger, and thus brighter, particles is still a drawback. There are fears that in presence of a large (several tens of nanometers) particle, the biological functions might be altered. Fortunately, new techniques are on the rise to eliminate the background and to perform alternative readout schemes and more sensitive detection. Another advantage is that nanodiamonds are good labels in a large number of imaging methods, which is vital for correlative microscopy. Despite the interest in diamond nanoparticles for new applications, many potential applications still await their implementation in vivo. Here quantum sensing applications, which allow nanoscale resolution magnetic resonance detection\[129\] or localized temperature measurements with milliKelvin accuracy,\[130\] are the most exciting developments which have recently been employed in cells but still await their in vivo use.

8. Future Perspective

In addition to the applications that are reviewed in this work, we would like to reflect on some of the rising fields, wherein nanodiamonds are expected to become increasingly important and the challenges that will have to be handled in the nearby future in order to bring NDs from the laboratory to the clinic.

8.1. Emerging Fields for ND-Based Medicine

Next to the numerous recent findings that have been made on drug delivery, this will probably remain an important field where nanodiamonds can be of use. The possibilities of nanodiamond-mediated drug delivery are not yet fully explored and more specifically, they could become very useful in upcoming fields that require highly efficient and safe drug delivery. Novel drug delivery approaches include combination therapy,\[11,21\] stem cell therapy,\[131,132\] and personalized medicine drug development.\[21,133\] Personalized in all cases, NDs can be functional in optimizing the effective drug delivery and high safety that are required for moving to the clinic.

Not only drug delivery, also gene and protein delivery and even delivery of genome editing tools could become ND-mediated. Genome editing is another field in medicine that is rising since the discovery of genome editing tools, of which the most famous and promising is the Crispr/Cas9 system. Nanodiamonds have been used to enhance the effectiveness of these tools.\[114\] More recently, the multimodal functionality of nanodiamonds has gained attention as well. This is the ability of NDs to function simultaneously in diagnostics, by tracking them to monitor internalization of a drug, on the one hand, and as therapeutic by using its drug delivery properties on the other hand.\[15,135\]

8.2. Translation to Clinic

The outstanding physical and chemical properties make nanodiamonds suitable for use as carriers of bioactive compounds in the clinic, but recent more generic reviews on nanomaterials in medicine agree that some important questions still have to be addressed before the translation of nanomaterials from the laboratory to the clinic can be made.\[13–15,21,131–133,135–137\] Although numerous studies have reported the excellent biocompatibility of NDs, safety remains one of the major concerns and understanding their metabolism is one of the remaining challenges. More pharmacokinetic and pharmacodynamics information is needed, as well as thorough understanding of the interaction between the nanocarriers and the physiological environment is needed. For example, the question whether NDs aggregate in the blood circulation and if this could have an effect on the drug performance should be answered. Next to determining its mode of excretion or accumulation in the human body, the long-term effects of ND-based treatments should be the subject of comparative studies. Systemic studies for long-term toxicity are needed to reach clinical trials. Mitragotri et al. proposed the need to set uniformity in preclinical trials.\[136\] Variability in applied particle sizes, surface modifications, model systems, and therapeutic doses have prevented systematic reviews. Performance of more consistent trials will enable systematic comparison of nanomaterial safety studies. Formation of a uniform protocol to characterize nanomaterials prior to clinical trials will also provide a common ground for nanomedicine testing and the regulatory approvals that are needed.\[136,137\]

Crucial in the translation process from laboratory to clinic is the collaboration between scientists from different fields, physicians, industrial researchers, investors, and regulatory authorities. The interdisciplinary nature of ND-based medicine requires this cooperation to ensure the expected clinical success. The cross-talk between scientists, regulators, and industry is essential for fast and safe development of nanomedicines and to promote translation of ND-based medicine into the market.\[137\] Although there is still a long road ahead and there are many remaining challenges for both the academic and industrial researchers, we are optimistic that the promise of nanodiamonds will be brought to reality.

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Conflict of Interest

The authors declare no conflict of interest.

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