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Coating nanodiamonds with biocompatible shells for applications in biology and medicine

Jitka Neburkova [a,b,1], Jan Vavra [a,1], Petr Cigler [a,*]

[a] Institute of Organic Chemistry and Biochemistry, v.v.i., Academy of Sciences of the Czech Republic, Flemingovo nam. 2, 166 10 Prague 6, Czech Republic
[b] First Faculty of Medicine, Charles University, Katerinska 32, 121 08 Prague 2, Czech Republic

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ABSTRACT

Use of nanodiamonds (NDs) as nontoxic nanoparticles for biological imaging, sensing, and drug delivery is expanding rapidly. The interest in NDs is triggered by their unique combination of optical properties. ND can accommodate nitrogen-vacancy color centers which provide stable fluorescence without photobleaching or photoblinking and their electronic structure is very sensitive to magnetic and electric fields. The limited options to control ND properties during synthesis or by direct surface functionalization leave room to be improved upon by employing surface coatings engineered precisely for a particular application. The major disadvantages of unmodified NDs are their limited colloidal stability and tendency to non-specifically adsorb biomolecules. This review aims to summarize recent advances in coating NDs (namely with silica and polymer shells), which addresses these disadvantages and enables the use of NDs in biological applications such as targeting of specific cells, drug delivery, and biological imaging.

1. Introduction

Nanodiamonds (NDs) are intensively studied as a platform for numerous applications in biology and medicine. This is partly due to their unique combination of optical properties unmatched by any other currently used fluorescent probe and partly due to their biocompatibility. Their extraordinary optical properties include almost unlimited photostability (no photobleaching), even under high power excitation [1]; the absence of photoblinking; and a long fluorescence lifetime [2–4]. Fluorescent nitrogen-vacancy (NV) centers within NDs give a broad emission peak, with a maximum at around 680 nm, which falls within the living tissue absorption window—an essential property for bioimaging applications [4,5]. Several sensing mechanisms utilizing NV centers in NDs have emerged recently, including optically detected magnetic resonance [6,7] and charge- and surface-responsive modulation of fluorescent spectra [8–10]. Exciting recently proposed applications involving NDs functioning in a biological environment include monitoring of ion channel function and real-time visualization of neural activity [11,12]. The main features of NDs have been summarized in a number of reviews, the most recent ones directed at ND properties and applications [13–15]; photophysics [6]; surface chemistry [16–18]; and the use of NDs in bioimaging [4,19,20], drug delivery [21], and nanoscale medicine [22].

Certain properties are required of nanoparticles (NPs) for deployment in biological systems; these include nontoxicity,

Abbreviations: ND, nanodiamond; NV, nitrogen-vacancy centers; NP, nanoparticle; HPHT, high pressure, high temperature; DND, detonation nanodiamond; TEOS, tetraethylorthosilicate; APTES, (3-aminopropyl)triethoxysilane; Tf, transferrin; PEG, polyethylenglycol; PG, polyglycerol; PPEGMA, poly(PEG methyl ether methacrylate); PHPMA, poly[*N*-(2-hydroxypropyl) methacrylamide]; BSA, bovine serum albumin; HSA, human serum albumin; PEI, polyethylene imine; PAH, polyallylamine hydrochloride; PDMAEMA, poly(2-(dimethylamino)ethyl methacrylate; BMA, butyl methacrylate; PBS, phosphate buffer saline; DOX, doxorubicin; ATRP, atom transfer radical polymerization; RAFT, reversible addition-fragmentation chain transfer; PMAA, poly(methacrylic acid); DLS, dynamic light scattering; ALKMA, propargylacrylamide; AZMA, 3-(azidopropyl)methacrylamide; TEM, transmission electron microscope; STEM, scanning TEM; HRTEM, high resolution TEM; EELS, electron energy loss spectroscopy; TfR, transferrin receptor; cRGD, cyclic RGD peptide.

* Corresponding author.
E-mail address: cigler@uochb.cas.cz (P. Cigler).
[1] These authors contributed equally.

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chemical and colloidal stability under physiological conditions, and a low level of nonspecific interactions with proteins, the so-called antifouling. While unmodified NDs are inherently nontoxic and chemically stable in biological environments, they tend to precipitate at physiological ionic strength, and proteins are readily adsorbed on the exposed surface. Elimination of these disadvantages is essential for bioapplications, because aggregates of NDs cannot be easily taken up by cells. These limitations can be overcome by the use of a so-called stealth coating made out of biocompatible polymers [23].

The main methods for production of NDs are detonation synthesis, chemical vapor deposition, and high pressure, high temperature synthesis (HPHT). Although the bulk structures of NDs of different origins are very similar, their purity, surface composition, and reactivity vary dramatically. The surface of detonation nanodiamonds (DNDs) contains a variety of functional groups, of which the abundant carboxyl group has been exploited the most [13,16]. A relatively new top-down approach of mechanically fracturing HPHT-synthesized microdiamonds with a proper content of interstitial nitrogen (100–200 ppm) [24] has the potential to create bright fluorescent NDs [25,26]. In this class of NDs, the surface content of carboxyl group is relatively low (~2% of the surface groups [27]); however, it is sufficient to coulombically stabilize colloids of these particles. The usual strategy to normalize the ND surface involves aerobic oxidation and oxidation in mineral acids, although even the normalized surface of HPHT NDs, which resembles the bulk diamond surface very closely, is vastly different from that of DNDs. The dominant surface group on HPHT NDs is the hydroxyl group [27]; thus, different strategies have to be adopted for surface functionalization of these particles.

2. Silica coatings on nanodiamonds

Amorphous silica can be used conveniently as a primary coating on any oxidized ND to normalize its surface, which enables the further use of well-investigated silane chemistry [28]. Most silication protocols utilize some modification of the well-known Stöber process [29], in which amorphous silica is created by a polycondensation reaction between silanols and the diamond's surface alcohols. The silanols are created *in situ* by base-catalyzed hydrolysis of a silyl ether, often tetraethylorthosilicate (TEOS) or (3-aminopropyl)triethoxysilane (APTES). The Stöber silication process is typically carried out in ethanol as a solvent, with an aqueous solution of ammonia acting as both catalyst (ammonia) and reagent (water). While the colloidal stability of NDs in ethanol is usually good, the increased ionic strength induced by the addition of ammonia causes ND particles to slowly aggregate and precipitate. Different approaches have been developed to overcome this phenomenon, including the adsorption of nonionic surfactants [30] or a water-soluble polymer, such as polyvinylpyrrolidone [31,32], onto ND particles prior to silica coating and the use of small unilamellar vesicles that encapsulate individual ND particles during silication [33]. Once a homogenous silica shell has been created, the dispersion of particles in the reaction mixture is stabilized, and the silanized particles are able to withstand a higher ionic strength than unmodified NDs without precipitating [28,34,35].

The first work utilizing silanization to form a primary layer on DND particles was published in 2006. DND aggregates with a diameter of 100 nm were functionalized with (3-aminopropyl)tri methoxysilane in dry acetone, which led to further aggregation and resulted in particles with a mean diameter of approximately 500 nm. The DND aggregates underwent borane reduction prior to coating, which contributed to their decreased colloidal stability during the APTES modification step. The introduction of terminal amino groups on the surface enabled peptide synthesis directly on the particles [36]. The same strategy was used to graft biotin molecules onto DNDs [37]. Great advancements in deagglomeration of DND particles have been achieved by beads-assisted sonication [35]. The individual deagglomerated DND particles, ranging in diameter from 4 to 10 nm, have a strong tendency to form aggregates again. Silanization with an acrylate-modified silane can be employed during beads-assisted sonication to stabilize the dispersion of deagglomerated particles [38].

Functionalized silanes, such as APTES and 3-(trimethoxysilyl) propyl methacrylate (TMSPMA), which have only three available bonding sites for the polycondensation reaction, tend to create very thin silica shells (1–2 nm) [37] (Fig. 1A and B). Even when a mixture of functionalized silane and excess TEOS is used, the layer self-terminates, exposing the functional group to solution and sterically preventing further condensation of silanes [39,40]. Interestingly, the surface loading depends on the choice of alkoxy substituent. Use of (3-aminopropyl)trimethoxysilane resulted in more than 4-fold higher loading of silanes compared to APTES. This difference can be attributed to the methoxy groups being better leaving groups, enhancing the reactivity of the respective silane [35]. These compact layers enable the ND particles to retain their original shape, while covering the surface densely with groups that are easy to further functionalize, enabling exceptionally high surface coverage of peptides or polymers [36,39]. Aminophenylboronic acid grafted to DNDs has been used to capture glycoproteins, and the presence of an initial silane coating enabled greater surface loading of aminophenylboronic acid compared to direct modification. This led to a significant increase in the effectiveness of glycoprotein capture [41].

Silica-modified NDs have been used directly *in vitro* to compare the effectiveness of electrostatically governed and receptor-mediated endocytosis on HeLa cells [44]. Positively charged ND-NH_2 particles interact with negatively charged cell membranes, which results in comparable uptake of NDs modified with transferrin (Tf), even though Tf-modified NDs interact specifically with overexpressed receptors on the studied cells. This work also revealed significantly decreased cell proliferation when the cells were exposed to aminosilica-modified NDs and ND-Tf particles. Unmodified carboxylated NDs had no effect on cell growth at the concentration used. Negatively charged or neutral NDs are better-suited to avoid non-specific interactions with cells [44].

The thickness of the silica coating can be controlled by varying the synthetic parameters. As the thickness of the silica layer increases, the shape of the composite particle changes from the irregular and spiky surface [45] of the core ND to almost spherical (Fig. 1D). A silica layer modifying the curvature of individual ND particles has been used as a model system for studies of cellular uptake of NPs [43]. An interesting phenomenon in which the cellular fate of NPs is governed primarily by their shape has been observed. Upon endocytosis, rounded particles tend to stay in endosomes, later move to lysosomes, and exit the cells by exocytosis, whereas particles with high curvature tend to penetrate the endosomes and accumulate in the cytoplasm. This profound effect of curvature been observed for all studied NPs, regardless of the surface chemical composition. This phenomenon was later exploited for efficient cytosolic gene delivery [46].

After the silica shell is grown, NPs are purified to remove the excess ammonia and reaction byproducts. However, once the free silanes are removed from the dispersion of silanized NDs, the polycondensation reaction will proceed in reverse, hydrolyzing the silica shell if water is present. Thus, it is advisable to store silica-coated particles in methanol or another polar anhydrous solvent. The dissolution rate of amorphous silica is further increased at higher ionic strength [47,48]. Weakly crosslinked functionalized silanes are even more prone to hydrolysis than TEOS-based silica.

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