A Nanodiamond is Forever: Functionalizable fluorescent nanodiamonds that do not photobleach or blink for in vitro and in vivo imaging

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ABSTRACT

Fluorescent nanodiamonds (NDs) are a new category of nanoparticles with optical properties that make them superior imaging probes that could replace commonly used fluorescence agents (i.e. dyes and quantum dots). In particular, their near infrared fluorescence makes them ideally suited for imaging in patients. However, ND use has been limited thus far because of difficulty in functionalizing or coating their inert surface and because of their tendency to aggregate. To solve these issues, we have coated nanodiamonds with silica using an innovative liposome-based coating process. Having such an optically superior, stable, and functionalizable nanoparticle essentially introduces a new building block to the fields of nanotechnology and nanomedicine, providing for a plethora applications. We have illustrated the unique features and potential uses of NDs in multiple applications: (1) high spatial and temporal resolution 3-D tracking over extended periods of time, (2) as stable fiducial markers for ultra high resolution microscopy across multiple wavelengths, and (3) wide-field background-free imaging through magnetic modulation of ND emission combined with computational lock-in signal recovery.

Keywords: near infrared imaging probe, nanoparticles, optical imaging, nanodiamonds, background-free imaging

1 BACKGROUND

Fluorescent nanodiamonds (NDs) are biocompatible particles with indefinite photo-stability that makes them superior imaging probes that could replace commonly used optical agents for a wide range of applications. The negatively charged nitrogen-vacancy (NV^{-}) center is a

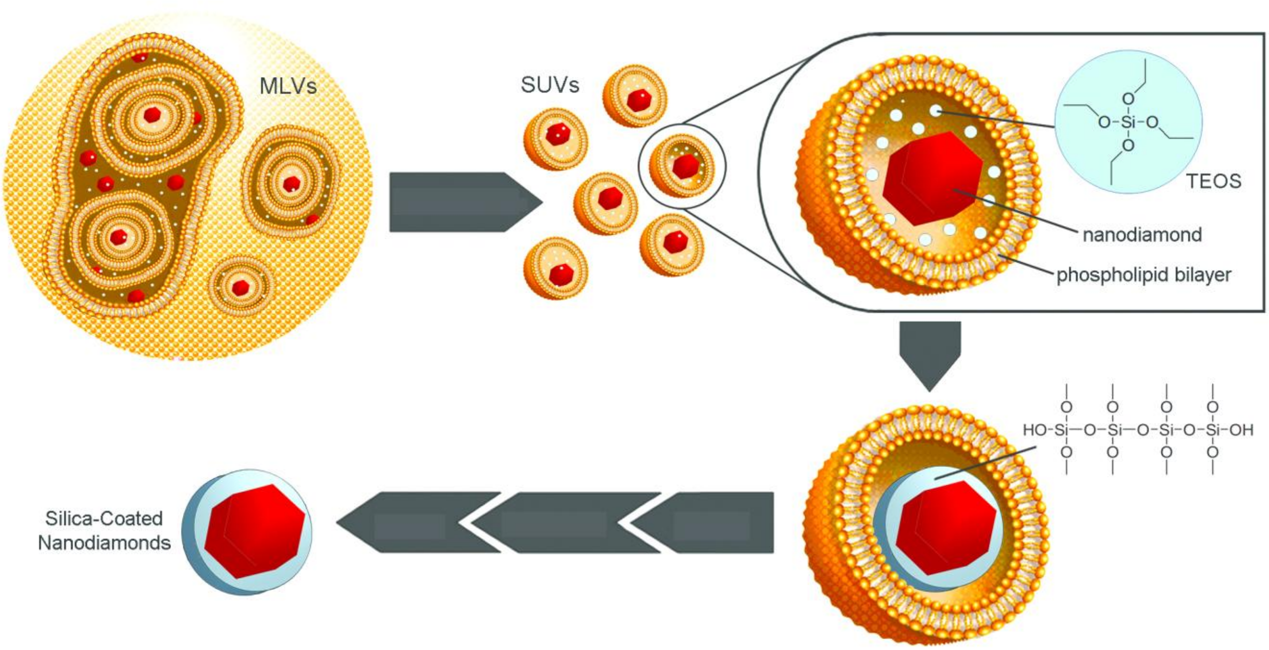

Figure 1: Nanodiamond encapsulation in silica. The diamonds and the islane precursor are trapped in multi-laminar vesicles that are then converted to small unilamilar vessicles or liposomes. The silane reaction to silica is then catelyzed, trapping the dimaonds in thin layers of silica. Finally, the liposome is washed away.

defect in the diamond lattice consisting of a substitutional nitrogen and a lattice vacancy that form a nearest-neighbor pair. NV^{-} centers are fluorescent sources with remarkable optical properties including quantum efficiency near unity, indefinite photo-stability (i.e. no photo-bleaching or blinking), broad excitation spectra, and exquisitely sensitive magnetic field-dependent fluorescence emission. Additionally, they have long fluorescence lifetimes (~17ns) that can be used for time-gated imaging to suppress background signal from tissue autofluorescence (1-2ns) [1]. In particular, their near infrared fluorescence makes them ideally suited for *in vivo* imaging [2-5]. Nonetheless, FND use has been not reached its potential because of difficulty in functionalizing their inert surface and because of aggregation issues.

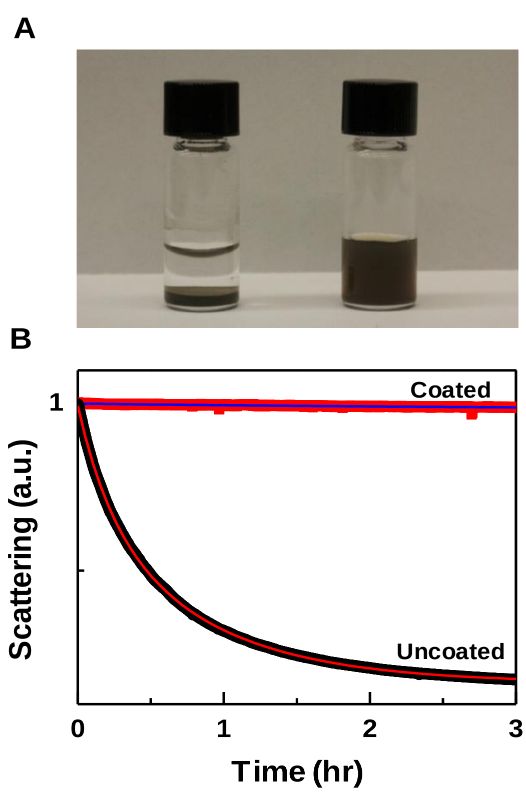

Figure 2: (A) Uncoated diamond in water (left vial) and silica-coated diamond in water (right vial). (B) Settling of uncoated (black circles) and silica-coated diamond (red square) measured by light scattering. Samples were excited with 635 nm and the scattering was measured at 90°. Settling of uncoated diamond was best fit (red line) by double exponentials, whereas that of coated diamond was best fit (blue line) by single exponential..

Silica helps in maintaining stability for particle suspensions during changes in pH or electrolyte concentration. Its silanol groups make the surface lyophilic [6]. The material is resistant to microbial attack, non-toxic, and biocompatible [7-8]. It is dispersible in aqueous and non-aqueous solutions and is resistant to swelling with changes in solvent polarity. Furthermore, silica is optically transparent [9]. which allows excitation and emission light to pass through the silica matrix efficiently. Moreover, the surface of silanol groups easily reacts with alcohols and silane coupling agents [10] for strong covalent bonding with a variety of ligands, including targeting agents such as antibodies and peptides, as well as sugars, nucleotides, and chelates.

2 COATING WITH SILICA

To overcome the difficulty of direct functionalization, we have coated nanodiamonds with silica using a liposome-based coating process (Figure 1). This renders the particle surface biocompatible, stable, and easy to perform additional chemistry with. Furthermore, the method self-selects for a desired particle size and produces a monodisperse agent. The coated NDs were colloidally stable, while the uncoated NDs immediately fell out of solution (Figure 2). Our coated NDs have thus far remained in solution for months.

3 APPLICATIONS

The nanodiamonds have a fluorescent signal that never photobleaches or blinks (Figure 3a). Thier fluorescence can also be modulated by an applied magnetic field (Figre 3d). Having such an optically superior, stable, and functionalizable nanoparticle essentially introduces a new building block to the fields of nanotechnology and nanomedicine, providing for a plethora applications. For instance, silica-coated NDs can be functionalized for multi-modal imaging with radiolabels and MRI contrast agents and targeted drug delivery with tracking ability. In fact, we have covalently attached functional moieties, such as biotin to NDs. We illustrate the unique features and potential uses of NDs from with several applications: (1) high spatial and temporal resolution 3-D tracking over extended periods of time (Figure 3b), (2) as stable fiducial markers for ultrahigh resolution microscopy across multiple wavelengths (Figure 3c), and (3) wide-field background-free imaging through magnetic modulation of ND emission (Figure 3d). With the success of demonstrating background-free imaging in a micropscopy setup, we have begun collecting preliminary results *in vivo* in mice injected with NDs.

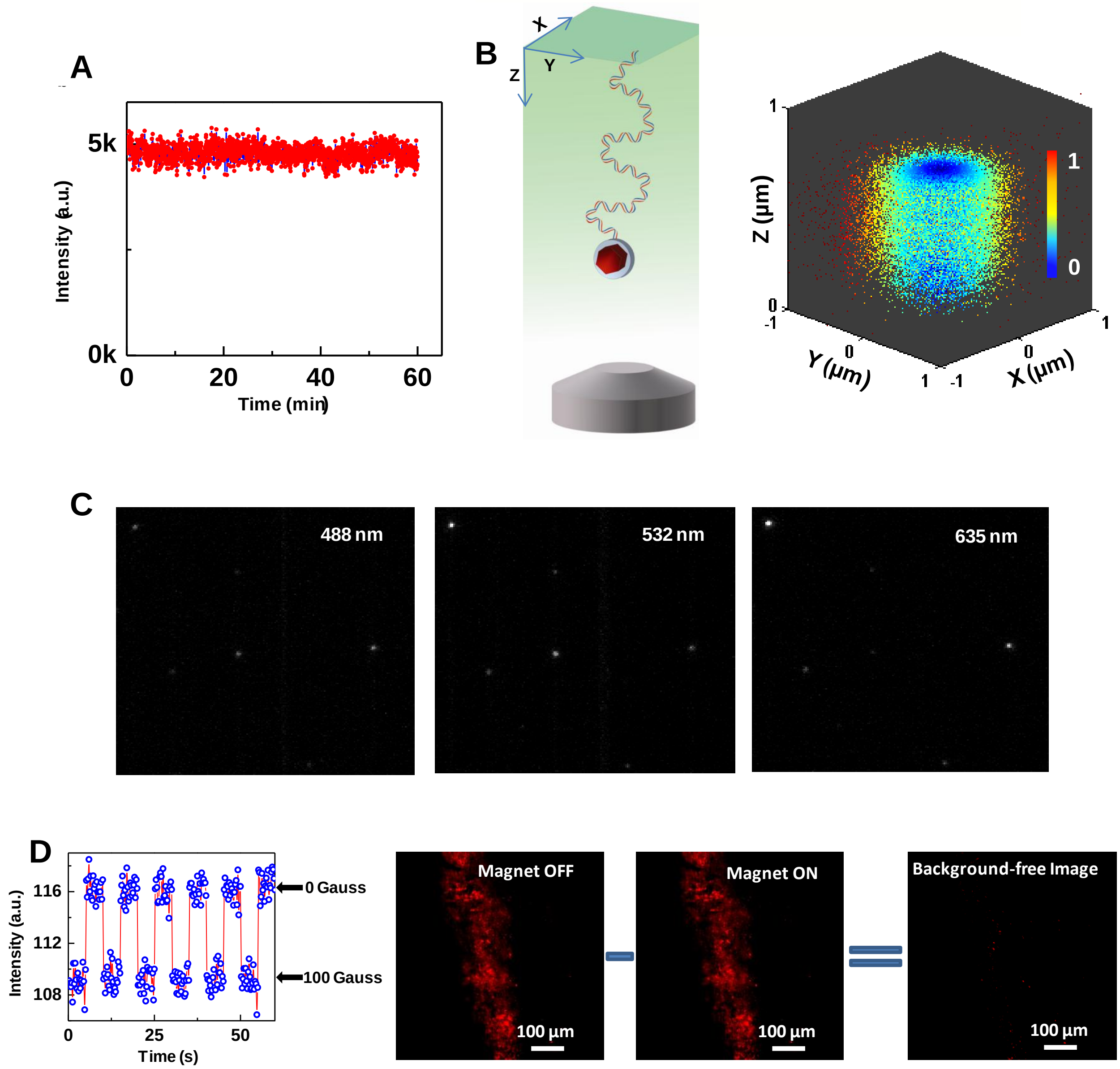

Figure 3: Applications of fluorescent nanodiamonds (A) NDs have an infinite fluorescent signal that does not photobleach or photoblink, as is seen in the emissions from a single ND recorded for over an hour. (B) The free 3-D motion of a DNA strand tethered to a glass slide was tracked using a ND that was attached on the free end. The schematic represents the setup in total internal relection microscopy and the X-Y-Z plot of data points recorded over a period of one hour. (C) Given their broad excitation spectrums, NDs can be used as fiducial markers for multicolor imaging. The same frame acquired with 1 ms exposure time at 488 nm, 532 nm, and 635 nm. (D) ND fluorescence signal modulates with a field of 100 gauss. This property can be maniputed to obtain background-free images by subtracting images with the magnetic field on from those with it off.

CONCLUSIONS

In summary, we have developed a simple method to encapsulate nanodiamonds with silica that stabilize them and enables further functionalization. We have demonstrated the utility of this approach by covalently linking the NDs to DNA for 3D tracking of an individual DNA molecule with high spatial and temporal resolution over an extended period of time. We have also used them as stable fiducial markers for ultra-high resolution microscopy across multiple wavelengths and for background-free imaging using magnetic modulaiton. Optically superior, stable, and functionalizable FNDs have a plethora of applications in the fields of nanotechnology and nanomedicine.

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