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Influence of surface composition on the colloidal stability of ultra-small detonation nanodiamonds in biological media

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ABSTRACT

Fluorescent nanodiamonds (NDs) are strong contenders as bio-labels for life science imaging, diagnostics and therapeutics. Ultimately, for their use in biomedical applications, their size should ideally be < 10 nm. Yet, even more critical for their specificity and efficient uptake in cellular systems, is their resilience to aggregation, which is dictated by their colloidal stability in complex, physiological environments. To this end, we characterize small detonation NDs (~5 nm) by examining their surface chemical profiles and stability in solutions of varying ionic strength and pH. Using dynamic light scattering measurements, we demonstrate that small monodisperse ND particles with chemically homogeneous and negatively charged surface profiles are more stable than positive particles under a broad range of simulated biological environments. We show that the colloidal stability of small clusters of both positive and negative detonation NDs is improved by functionalization with bovine serum albumin. Based on these analyses, we propose and describe strategies for enhancing the overall colloidal stability of detonation NDs and their resilience to aggregation. Our findings provide a practical framework towards the reduction in size of the bio-conjugates employed to probe complex biological systems, and the advancement of bio-imaging techniques with minimal perturbation of the molecular trafficking in cellular and organelle systems.

1. Introduction

Fluorescent nanodiamond (ND) particles are exceptional objects which are being increasingly adopted in several biomedical applications including drug delivery, imaging and diagnostics [1–3]. In the field of biological imaging, their low cytotoxicity, high quantum yield, long fluorescence lifetime and stable photoluminescence sets them apart from many other competitive systems currently employed as bio-labels [4–6]. The quest for bio-imaging at the molecular level has led to research on luminescent nitrogen vacancy defects in small diamond particles with high-pressure high-temperature (HPHT) nanodiamonds (> 10 nm) the most widely used nanoparticle with notable fluorescent defect properties (as recently reviewed by [7]). Detonation nanodiamond (DND) particles of about 5 nm in size also display fluorescence properties [8–11]; although their use for biological applications, including bio-imaging, is not yet as widely reported. One notable challenge with using either HPHT or detonation NDs as bio-labels is their strong and undesirable tendency to aggregate while in complex physiological environments.

When nanomaterials enter biological environments, the biomolecules – mostly proteins – compete for binding to the surface of the nanoparticle [12–14], forming a protein corona layer around it. The proteins which are most abundant are the first to become adsorbed onto the surface. Over time, they are replaced by proteins which have a higher affinity to the nanoparticles surface [15]. This is known as the Vroman effect. A recent study showed that, while the amount of proteins around the nanoparticle may change over time, the composition of the protein corona remains essentially unchanged [16]. This indicates that the biomolecular layer around the nanoparticle is most likely stable in composition: it is instead the interaction at the nanoparticle bio-interface that dominates the binding dynamics in a complex and non-trivial way.

The biological behaviour of nanoparticles in solution is ultimately determined by their physico-chemical properties and – markedly –

Abbreviations: ND, nanodiamond; BSA, bovine serum albumin; HSA, Human serum albumin; DND, detonation nanodiamond; DI, deionized; FTIR, Fourier transform infrared spectroscopy; DLS, dynamic light scattering

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these tend to depend on many factors including surface chemistry profile of the nanoparticles, presence of salts, and pH of the surrounding solution. This is important as the behaviour and fate of nanoparticles is too often overly idealised in practical situations, such as cellular systems, where it can be difficult to anticipate the effect of complex environmental conditions on the colloidal behaviour of the nanoparticles. Strategies to improve the stability of nanoparticles do exist, yet they tend to compromise the functioning efficiency of the nanoparticle with respect to target molecules [17]. Further, the alteration in the surface profile of nanoparticles in physiological conditions may not only lead to compromised functionality but also their aggregation [18–22].

In context of nanodiamond particles; methods to control their size and surface functionalisation are readily available, but effective approaches to manipulate their colloidal stability are lacking. This is especially true for ultra-small NDs, and detonation ND in particular where strongly bound agglomerates usually form if no countermeasures are taken [23]. In general, the colloidal stability of NDs is electrostatically stabilised by increasing the surface charge density, which subsequently results in increased protein adsorption [21,24]. The surface functionalisation of nanodiamonds by oxidation [25], or by binding blocking agents such as bovine serum albumin (BSA) [26] and polyglycerol [27], have all been shown to change the charge density, as well as increase the chemical homogeneity of the NDs' surface.

Surface functionalisation approaches have worked particularly well for large HPHT nanodiamonds. In contrast, methods to achieve a stable monodisperse suspension of primary particles (4–5 nm) of small detonation nanodiamonds are not as well established, with most studies reporting only on conditions which render the stability of large flocculating detonation ND dispersions of sizes greater than ~50 nm [21,28,29]. Additionally, any methods used to improve stability of a monodisperse suspension must also be biocompatible for intended biomedical environments and applications [30]. For example, HPHT synthesized NDs, purified by air oxidation and strong oxidative acid treatments to remove all contaminants and graphitic surface layers show excellent hemocompatibility [31] – a prerequisite if they are to be used safely in biomedical contexts.

In this work, we present a systematic study of the role of the changing environment of surface functionalisation parameters on the overall colloidal stability of NDs in aqueous suspensions. We focus specifically on detonation NDs which are most promising for bio-applications because of their small size (< 10 nm). Small NDs have a higher mobility and so can more readily extravasate and be tracked through biological ultrastructures that are notoriously difficult to study such as endothelium in inflammatory sites, the epithelium of the liver and intestinal tract, perivascular spaces in the spinal cord, tumors, or microcapillaries. Drug release from nanoparticles is also affected by particle size. Smaller particles have greater curvature of their surface which results in association of drugs, or other biomolecules, at or near the particle surface, leading to faster drug release. The large surface area-to volume ratio also decreases the protein-protein interactions among the biomolecules adsorbed on the nanoparticle's surface, resulting in fewer conformational changes in the attached proteins [30] Together, the high mobility and high surface-to-volume ratio of detonation NDs (also optimal for surface functionalisation) makes them ideal candidates for in vivo bio-imaging and drug delivery – provided that their colloidal stability and dispersibility are preserved upon entering biological systems [32,33]. This is relevant, as the very same high surface-to-volume ratio makes detonation NDs more susceptible to aggregation in the presence of salt [34] and other bio-components in the immediate environment.

Small NDs are usually produced by detonation [35], and typically form tight clusters which can only be de-aggregated through specific approaches including sonication, fractionation by centrifugation [36], plasma treatments [37,38], high-energy ball milling [39,40], thermal oxidation [41] or chemical processes [11]. The colloidal stability for large clusters (size ~30–60 nm) of small detonation NDs with primary particle size of ~5 nm has been previously studied over a range of pH values through their zeta potential variation [29]. However, an in-depth analysis has never been conducted for individually-dispersed detonation NDs. Here we present a systematic analysis of the factors that lead to improved dispersibility and colloidal stability of monosized detonated nanodiamonds (DNDs), which are ~5–6 nm in size, in complex physiological media. We use dynamic light scattering (DLS) measurements to determine the aggregation tendency of the isolated monodispersed DND particles of different surface chemical profiles in solutions over a broad range of pH values (2 to 12) and ionic strengths (up to 1 M NaCl). Our findings suggest that the ND surface homogeneity plays a key role on the colloidal stability of the DND nanoparticle. Specifically, we find that when DNDs are used in complex media of high ionic strength such as phosphate buffer saline (PBS) – a commonly used isotonic buffered salt solution – the colloidal stability is significantly reduced. Finally, we propose and discuss strategies, such as surface functionalisation of the DNDs, to improve their overall stability and resilience to aggregation.

2. Experimental

2.1. DND material and surface characterization (FTIR & XRD)

Positively and negatively charged detonation nanodiamond (DND) colloidal suspensions (ND-H2O-5P, ND-H2O-5N, respectively), of average particle size of ~5 nm in water, were obtained from Adámas Nanotechnologies (Raleigh, North Carolina, USA). The DND solutions (10 mg/mL) were bath-sonicated for 30 min prior to use.

To characterize the surface groups by attenuated total reflectance infrared spectroscopy (ATR-FTIR) using a Nicolet™ iS10™ (Thermo Scientific), the positive and negative DND samples were prepared by drying ~10 mg of the ND powder in a hot-air oven at 100 °C for 30 min. FTIR spectra were collected under nitrogen to minimise the effect of atmospheric carbon dioxide. FTIR spectral analysis was performed within the wave number range 500–4000 cm^{-1} (total scans 512, resolution 4 cm^{-1}). The internal reflection element used was a diamond HATR crystal of the Thermo Scientific™ Smart iTR™ ATR Accessory.

X-ray diffraction (XRD) measurements were also conducted on the DND powders. The samples were deposited on a low-background silicon holder via evaporation, and analysed using a Bruker D8 Discover diffractometer using Cu Kα radiation (λ = 1.5418 Å) at 45 kV and 35 mA. The diamond (111) peak was used to analyse peak broadening in order to determine the crystallite size using Scherrer equation. Experimental XRD data were fitted using the software package High Score Plus (version 3 PANalytical). An internal standard was used to calibrate peak broadening.

2.2. DND quantification of colloid (DLS & zeta potential)

A 2 mg/mL sample of the positive or negative DND particles were prepared in deionized (DI) water or potassium chloride (KCl) solutions of increasing concentration (1×10^{-5}, 1×10^{-4}, 1×10^{-3}, 1×10^{-2}, 1×10^{-1} and 1.0 M). The DND suspensions were left at room temperature for 48 h before measurement. All samples were then centrifuged by ultracentrifugation at 40,000*g* for 8 h in a fixed angle rotor (TLA 110 rotor, Optima MAX-XP Ultracentrifuge, Beckman Coulter) to remove large aggregates. Following ultracentrifugation, UV–Vis spectrophotometry was used to obtain an estimate of the amount of monodispersed DNDs retained in solution relative to the amount of DNDs remaining in suspension for the DI-water-only sample. The absorbance of the solution at 400 nm was measured using a NanoDrop 100 spectrophotometer (Thermo Fisher Scientific). A relative yield was reported for samples under the conditions of varying salt concentrations.

The particle size distributions and zeta potentials for all DND