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Fabrication of blue-fluorescent nanodiamonds modified with alkyl isocyanate for cellular bioimaging

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ABSTRACT

This paper describes the fabrication of water-dispersible nanodiamond (ND) clusters with blue fluorescence for cellular bioimaging. Poly(ethylene glycol) carboxyl methyl acid (mPEG-COOH) and alkyl isocyanates with different chain lengths were conjugated onto the surface of the ND clusters for water dispersibility and fluorescence via carbodiimide chemistry. The relative fluorescence intensity was increased with the increases in the chain length of alkyl isocyanate and also their conjugated concentration. The ND clusters (average size of 37.6 nm and zeta potential of 26.6 mV) with mPEG-COOH and octadecyl isocyanate (ODI) emitted relatively higher blue fluorescence intensity under excitation at 350 nm as well as favorable water dispersibility. After cellular uptake of the ND clusters, blue fluorescence inside the cells was confirmed by confocal laser scanning microscopy. The ND clusters conjugated with mPEG-COOH and ODI can potentially be used for cellular bioimaging.

1. Introduction

Multi-functional nanoparticles are of great importance in various biomedical applications [1–6]. Among many potential nanomaterials, carbon-based nanoparticles such as nanodiamonds (NDs), fullerenes (C60), carbon nanotubes (CNTs), and graphene have been widely investigated in biomedical fields including nanomedicine, tissue engineering, gene delivery, and biosensors [7–11]. Recently, NDs with individual diameters of 2–10 nm and truncated octahedral morphologies have attracted much attention as promising nanomaterials because they offer near-spherical geometry, high mechanical and optical properties, high surface areas, and excellent biocompatibility [12]. Additionally, ND surfaces can be readily modified with various functional groups for either covalent or noncovalent conjugation with biomolecules; they are thus well suited for various biomedical applications such as drug delivery, tissue engineering, bioimaging, and theranostics [13].

Recently, the application of NDs was expanded to molecular and in vitro/in vivo bioimaging. Ion beam irradiation was mainly employed to produce fluorescent NDs (FND) [14,15]. The FNDs with nitrogen-vacancy defect centers were prepared by irradiation with a proton beam and subsequent annealing, which had a red emission [15]. The FNDs have been considered as attractive nanoplatforms for bioimaging because they offer unique photophysical features such as green or red emissions, long fluorescence lifetimes, and excellent photostability [16–18]. However, the ion beam irradiation method is limited by complex experimental processes and expensive equipment requirements.

On the other hands, Mochalin et al. fabricated blue fluorescent NDs by simply conjugating octadecylamine (ODA) onto the surface of NDs via amide bond [9]. They assumed that the mechanism of blue fluorescence could be due to the polyaromatic structure formation of ODA. Although the fluorescence mechanism of the ODA-conjugated NDs is not fully understood, the surface modification method can be another facile route for the production of NDs with fluorescent property. The ODA-conjugated NDs were not dispersed in water phase due to the intrinsic hydrophobicities of NDs and ODA [19,20], possibly limiting their applications in cellular bioimaging. In this work, ND clusters were conjugated with non-fluorescent alkyl isocyanate (ISO) and poly(ethylene glycol) carboxyl methyl acid (mPEG-COOH) for water-dispersible fluorescently labelled NDs. We also studied the effect of the chain length and concentration of ISO on the optical properties. The proposed ND clusters modified with ISO and mPEG-COOH can be a potential imaging agent for cellular imaging.

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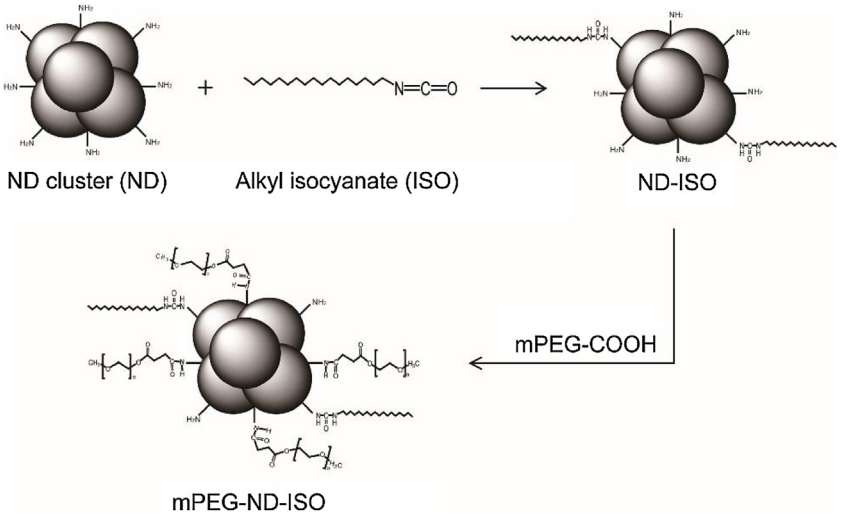

Scheme 1. Schematic of synthesis of mPEG-ND–ODI. The amine groups of ND were sequentially conjugated with alkyl isocyanate via urea bond and mPEG-COOH via amide bond.

2. Material and methods

2.1. Preparation and characterization of ND clusters

Oleylamine-modified NDs (Neomond, Bucheon, Korea) dispersion (30 mg/15 mL anhydrous N,N-dimethyl formamide (DMF, Sigma-Aldrich, St. Louis, MO, USA)) was sonicated using an ultrasonicator (Sonics and Materials Inc., Danbury, CT, USA) in an ice bath for 30 min and stirred for 24 h at room temperature. Hexyl isocyanate (HI, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), octyl isocyanate (OI, Sigma-Aldrich), decyl isocyanate (DI, Sigma-Aldrich), dodeccyl isocyanate (DDI, Sigma-Aldrich), tetradecyl isocyanate (TDI, Santa Cruz Biotechnology, Dallas, TX, USA), and octadecyl isocyanate (ODI, Sigma-Aldrich) were used to conjugate NDs and ISO. For conjugation, 1 mM of ISO in 5 mL of DMF was added into the ND dispersion and the mixture was stirred for 24 h at room temperature, followed by dialysis and freeze-drying to obtain NDs modified with ISO (referred to ND–ISO). Monofunctional poly(ethylene glycol) carboxyl methyl acid (mPEG-COOH, average Mn ~2000, Creative PEGWorks, Chapel Hill, NC, USA) was conjugated to the ND–ISO by amide bond formation with 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM, Tokyo Chemical Industry Co., Ltd.). 70 mg of DMTMM was added to 0.5 g of mPEG-COOH in 5 mL DMF and stirred for 24 h at room temperature. NDs modified with ISO and mPEG-COOH (referred to mPEG-ND-ISO) were obtained after 2 days of dialysis. To analyze each chemical reaction among ND, ISO, and mPEG-COOH, ND, ISO, mPEG-COOH, ND-ISO, mPEG-ND-ISO were examined by Fourier Transform Infrared Spectroscopy (FT-IR, Bruker Optics Ltd., Billerica, MA, USA). The resulting mPEG-ND-ISO was analyzed with a zeta potential/size analyzer (Malvern Instruments Ltd., Worcestershire, UK) and an ultraviolet–visible (UV/vis) spectrophotometer (PerkinElmer, Norwalk, CT, USA). The morphology of the mPEG-ND-ISO was characterized by scanning electron microscopy (Hitachi, Tokyo, Japan). To evaluate colloidal stability, ND, ND-ODI, and mPEG-ND-ODI (0.1 wt%) were dispersed in deionized water (D.W.) and their zeta potentials and sizes were measured for 21 days. In addition, the size and zeta potential of mPEG-ND-ODI (0.1 wt%) were measured after mixing with fetal bovine serum (FBS, 10%) solution for 24 h.

2.2. Fluorescence intensity

Aqueous dispersions (0.1 mL) of ND and mPEG-ND-ISO were added to each well of a 96-well plate. The relative fluorescence intensity (RFU) spectra were measured with a fluorescence spectrophotometer using a 150-W Xe lamp (Biotek, Winooski, VT, USA). The emission spectra were recorded in the wavelength range of 600–350 nm upon excitation at 320 nm. The slot width of the emission was 10.0 nm.

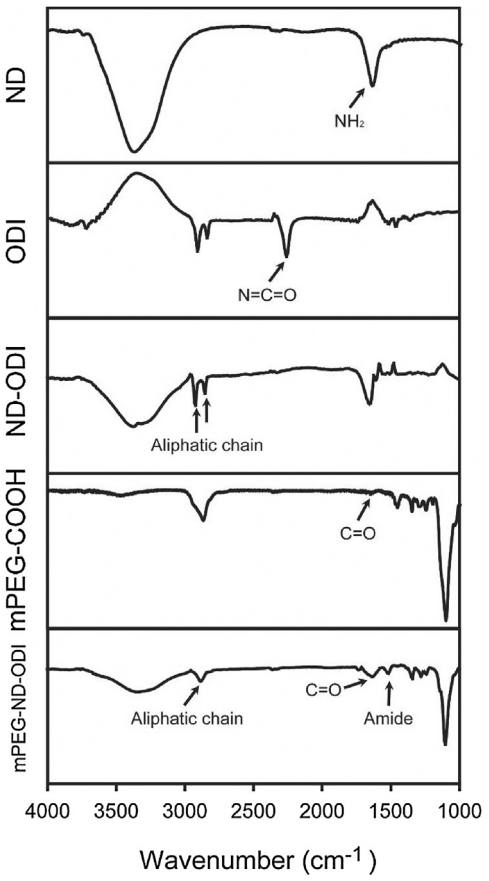

Fig. 1. FT-IR spectra of ND, ODI, ND-ODI, mPEG-COOH, and mPEG-ND-ODI.