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Microwave-assisted efficient conjugation of nanodiamond and paclitaxel

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ABSTRACT

Nanodiamond has recently received considerable attention due to the various possible applications in medical field such as drug delivery and bio-labeling. For this purpose suitable and effective surface functionalization of the diamond material are required. A versatile and reproducible surface modification method of nanoscale diamond is essential for functionalization. We introduce the input of microwave energy to assist the functionalization of nanodiamond surface. The feasibility of such a process is illustrated by comparing the biological assay of ND-paclitaxel synthesized by conventional and microwave irradiating. Using a microwave we manage to have approximately doubled grafted molecules per nanoparticle of nanodiamond.

Nanodiamond (ND), commercially produced by detonation has emerged to be new class of nanomaterial. Recently, the uptake of ND by living cell found in the biological ND research facilitated the use of NDs as drug carriers and delivery vehicles.[1–6] NDs possess numerous hallmarks of an ideal drug delivery system and are promising materials for advancing cancer therapy. It has been observed that nanodiamond did not induce cytotoxicity,[7–11] and the body's immune system does not attack them. They can bind tightly to a variety of molecules and deliver them right into a tumor. It is therefore of great interest, and high feasibility to modify the surface of NDs using organic entities to compound with substrates.[12,13] For the use of nanoparticles in biological systems it is necessary to modify the particle surface according to the specific requirements for the desired application. Hence it is important to find methods to functionalize the diamond surface with bioactive moieties.[14] Recently, we reported the synthesis of higher chain alcohols for manipulation of different functional groups such as peptides for the covalent attachment of drug molecules, and chiral ligands for asymmetric aldol reactions.[15] We also reported the covalent linkage of paclitaxel onto such diamond nanoparticles by means of bifunctional linker molecules such as alkyl chains with terminal amino groups for drug delivery in cancer therapy.[16] Aggregation behavior of nanodiamond affects the functionalization of nanodiamond surface and reduces the amount of conjugated compound on the surface. Also, a precise control of the number and orientation of the immobilized molecules on nanoparticles is easy to obtain.

Microwave irradiation has gained importance in the past decade as a powerful tool for rapid and efficient synthesis of a variety of compounds.[17,18] The application of microwave irradiation to provide enhanced reaction rate and improved product field in chemical synthesis and it is providing quite successful in the formation of a variety of carbon—heteroatom bonds. Moreover microwave heating could be used in heterogeneous media and has shown its efficiency in solid support synthesis. In microwave application, heating is caused by the interaction of the permanent dipole moment of the molecule with the high frequency electromagnetic radiation. In comparison with conventional heating, this novel method shortens reaction time by a factor of approximately 20. Also, heating is not only quick but also uniformly spread through the entire bulk of the reaction mixture. This may result in narrow particle size distribution. Recently microwave-assisted carboxylation of nanodiamond have been studied and observed that ND core was unaffected by microwave radiation.[19] In the present research, we wished to exploit the potential of the microwave technique to reduce the reaction time, and possibly improve the coupling processes of biomolecules on the nanodiamond surface. Efficiency of microwave assisted synthesis was examined by coupling and decoupling of stearic acid with intermediate ND-alcohol. The amount of stearic acid loaded on the surface of nanodiamond by microwave treatment and classical heating mode is compared.

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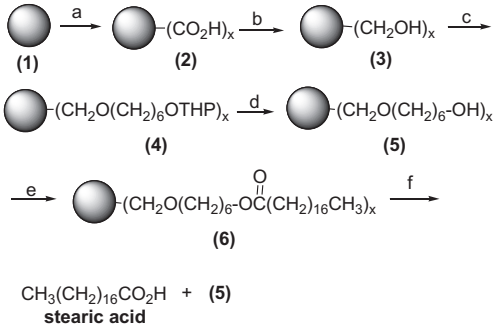
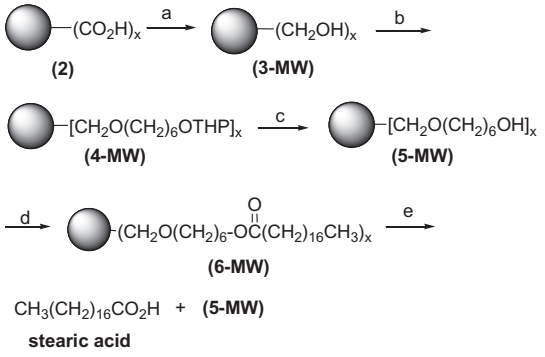
Initial chemical treatment of ND powders (**1**) for carboxylation was carried out according to standard procedure[15,16] (Scheme 1). The ND particles were stirred in a 3:1 (v/v) mixture of concentrated HCl and HNO_3 at room temperature for three days, then diluted with deionized H_2O and separated by centrifugation at 900 rpm. After centrifugation, the pellets were extensively rinsed with deionized H_2O three times. Thereafter, ND particles were heated in 0.1 M NaOH solution at 90 °C for 2 h. The ND particles were again heated in 0.1 M HCl at 90 °C for 2 h. The resulting carboxylated-ND (**2**) was dried under vacuum for 24 h. A mixture of ND-$(CO_2H)_x$ and THF was sonicated under argon for 5 min. After this time, $LiAlH_4$ was added, and the system was refluxed for 24 h. The reaction mixture was cooled to room temperature and quenched with deionized H_2O. The supernatant liquid was removed by centrifugation at 900 rpm, and the residue was rinsed with deionized H_2O three times. The residue was then heated in 6 M NaOH at 90 °C overnight. The reaction mixture was cooled to room temperature, washed, and treated with 0.1 M HCl as described earlier. The repeatedly-washed ND-$(CH_2OH)_x$ (**3**) was dried under vacuum at 50 °C. To the ND-$(CH_2ONa)_x$ mixture generated from (**3**) and NaH in THF was added 6-(chloro-hexyloxy)-tetrahydropyran, and the mixture was stirred at 45 °C for 24 h. The reaction mixture was cooled to room temperature, washed with THF and water as before, and finally dried under vacuum. A suspension of ND-$(CH_2O(CH_2)_6OTHP)_x$ (**4**) in MeOH/H_2O (3:1) was sonicated for 5 min; *p*-TsOH was then added until the solution became acidic, after which it was stirred at room temperature overnight. The reaction mixture was worked-up as described earlier to generate (**5**) as a dry powder. A suspension of ND-$(CH_2O(CH_2)_6OH)_x$ (**5**) and stearoyl chloride in THF was sonicated for 5 min; after which it was stirred at room temperature overnight. The reaction mixture was worked-up as described earlier to generate (**6**) as a dry powder. A mixture of (**6**) and 2 M NaOH in THF was sonicated for 3 min; after which it was refluxed overnight. The reaction mixture was cooled to room temperature and the supernatant liquid was removed by centrifugation at 900 rpm, and the residue was rinsed with THF and deionized H_2O three times. The solid phase was acidified by using 1 M HCl and was rinsed with THF and deionized H_2O three times. The combined liquid phase was acidified by 1 M HCl and extracted with EtOAc. The combined extracts were dried over anhydrous $MgSO_4$ and concentrated to give the stearic acid. The same procedure was repeated three times to give stearic acid average 33.4 mg (32.2, 32.2, 35.9 mg) from 1 g of (**6**).

A mixture of ND-$(CO_2H)_x$ (**2**) and $LiAlH_4$ in THF was refluxed using microwave (600 W, 55 °C) under argon for 2 h (Scheme 2). The reaction mixture was cooled to room temperature and quenched with deionized H_2O. The supernatant liquid was removed by centrifugation at 900 rpm, and the residue was rinsed with deionized H_2O two times. The residue was then heated in 6 M NaOH at 90 °C overnight. The reaction mixture was cooled to room temperature, washed, and treated with 0.1 M HCl. The repeatedly-washed ND-$(CH_2OH)_n$ (**3-MW**) was dried under vacuum at 50 °C. To the ND-$(CH_2ONa)_n$ mixture generated from **3-MW** and NaH in THF was added 6-(chloro-hexyloxy)-tetrahydropyran, and the mixture was refluxed using microwave (600 W, 55 °C) for 2 h. The reaction mixture was cooled to room temperature, washed with THF and water and dried under a vacuum to afford ND-$(CH_2O(CH_2)_6OTHP)_x$ (**4-MW**). To a suspension of **4-MW** in MeOH was added *p*-TsOH, after which it was agitated by microwave (30 W, 30 °C) for 2 h, and was stirred at room temperature overnight. The reaction mixture was worked-up to generate ND-$(CH_2O(CH_2)_6OH)_x$ (**5-MW**) as a dry powder. A suspension of **5-MW** and stearoyl chloride in THF was agitated by microwave (350 W, 50 °C) for 2 h, and was stirred at room temperature overnight. The reaction mixture was worked-up as described earlier to generate **6-MW** as a dry powder. Amount of stearic acid loaded on the surface was determined by saponification of **6-MW**. The same procedure was repeated three times to give stearic acid average 51.6 mg from 1 g of (**6-MW**).

The conjugation of ND and paclitaxel using microwave has been shown in Scheme 3. Triethylamine was added to a slurry of ND-$(CH_2O(CH_2)_6OH)_x$ (**5-MW**) in THF at 0 °C, and the mixture was stirred for 5 min. Methanesulfonyl chloride was then added dropwise, and the resulting mixture was stirred at 0 °C for 1 h and was agitated by microwave (30 W, 30 °C) for 2 h. Deionized H_2O was added, and the centrifuged residue was washed repeatedly with THF, water, and finally dried under a vacuum to yield ND-$(CH_2O(CH_2)_6OMs)_x$ (**7-MW**). ND-$(CH_2O(CH_2)_6OMs)_x$ (**7-MW**) was suspended in NH_4OH and was agitated by microwave (300 W, 70 °C) for 2 h. The reaction mixture was cooled to room temperature and rinsed with acetone. Separation by centrifugation at 900 rpm yielded ND-$(CH_2O(CH_2)_6NH_2)_x$ (**8-MW**), which was then dried under a vacuum. EEDQ was added to a solution of paclitaxel-2′-succinate (**9**) in dry CH_2Cl_2 and stirred for 30 min at room temperature.[20] To this solution was added a suspension of **8-MW** and Et_3N in CH_2Cl_2. The resulting mixture was agitated by microwave (25 W, 25 °C) for 2 h, and stirred at room temperature overnight. Paclitaxel-conjugated ND (**10-MW**) was separated by centrifugation at 900 rpm and then rinsed three times with CH_2Cl_2, three times with THF, and three times with deionized



Scheme 1. Chemical synthesis of ND-stearic ester. The chemical products are indicated by the numbers. Reagents and conditions: (a) HCl/HNO_3 (3:1), rt, 3 d; 1 M NaOH, 90 °C, 2 h; 1 M HCl, 90 °C, 2 h; (b) $LiAlH_4$, THF, reflux 24 h; 6 M NaOH, 90 °C, overnight; (c) NaH, THF, THP–O–$(CH_2)_5CH_2Cl$; (d) *p*-TsOH, MeOH/H_2O (3:1), rt, overnight; (e) $CH_3(CH_2)_{16}COCl$, THF, sonication 5 min, rt, overnight; (f) 2 M NaOH, THF, sonication 3 min, reflux 24 h.



Scheme 2. Chemical synthesis of ND-stearic ester. The chemical products are indicated by the numbers. Reagents and conditions: (a) $LiAlH_4$, THF, MW (600 W, 55 °C), reflux 2 h; 6 M NaOH, 90 °C, overnight; (b) NaH, THF, THP–O–$(CH_2)_5CH_2Cl$, MW (600 W, 55 °C) 2 h; (c) *p*-TsOH, MeOH, MW (30 W, 30 °C), 2 h, rt, overnight; (d) $CH_3(CH_2)_{16}COCl$, THF, MW (350 W, 50 °C) 2 h, rt, overnight; (e) 2 M NaOH, THF, sonication 3 min, reflux 24 h.