ACS APPLIED MATERIALS & INTERFACES

Research Article

www.acsami.org

Directly Thiolated Modification onto the Surface of Detonation Nanodiamonds

Ming-Hua Hsu,*[,†] Hong Chuang,[†,‡] Fong-Yu Cheng,[§] Ying-Pei Huang,[†,‡] Chien-Chung Han,[‡] Jiun-Yu Chen,[∥] Su-Chin Huang,[∥] Jen-Kun Chen,[∥] Dian-Syue Wu,[⊥] Hsueh-Liang Chu,[⊥] and Chia-Ching Chang[⊥,#]

[†]Nuclear Science & Technology Development Center and [‡]Department of Chemistry, National Tsing Hua University, Hsinchu 30013, Taiwan

[§]Institute of Oral Medicine, College of Medicine, National Cheng Kung University, Tainan 701, Taiwan

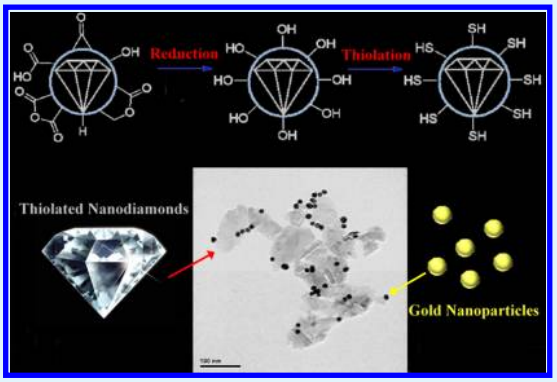
[∥]Institute of Biomedical Engineering and Nanomedicine, National Health Research Institutes, Miaoli 35053, Taiwan

[⊥]Department of Biological Science and Technology, National Chiao Tung University, Hsinchu 300, Taiwan

[#]Institute of Physics, Academia Sinica, Taipei 115, Taiwan

Supporting Information

ABSTRACT: An efficient method for modifying the surface of detonation nanodiamonds (5 and 100 nm) with thiol groups (−SH) by using an organic chemistry strategy is presented herein. Thiolated nanodiamonds were characterized by spectroscopic techniques, and the atomic percentage of sulfur was analyzed by elemental analysis and X-ray photoelectron spectroscopy. The conjugation between thiolated nanodiamonds and gold nanoparticles was elucidated by transmission electron microscopy and UV−vis spectrometry. Moreover, the material did not show significant cytotoxicity to the human lung carcinoma cell line and may prospectively be applied in bioconjugated technology. The new method that we elucidated may significantly improve the approach to surface modification of detonation nanodiamonds and build up a new platform for the application of nanodiamonds.



KEYWORDS: *thiolated, nanodiamond, detonation nanodiamond, surface modification, thiolated nanodiamond, functionalized nanodiamond*

INTRODUCTION

Nanotechnology has been applied in many fields of research, such as surface science, materials, semiconductor, imaging, and drug delivery. The use of nanoscale materials or devices in the diagnosis or treatment of diseases is termed nanomedicine.[1] Nanodiamond (ND), a carbon-based material, has attracted much attention in the past decade.[2] The nanoscale diamond was accidentally discovered by Russian scientists in the 1960s while studying diamond synthesis by shock compression.[3] The detonation nanodiamonds can be synthesized by exploding the trinitrotoluene−hexogen mixture in a closed metallic chamber; the shock wave will transform the graphite into nanodiamonds.[4] Detonation nanodiamond exhibits biocompatibility and low toxicity and is thus a promising candidate material for biological and medical applications.[4,5] Nanodiamonds can be applied for controlled drug-delivery applications. Ho and co-workers demonstrated that nanodiamond can be loaded and used for the delivery of chemotherapy drugs.[6−9] For specific purposes, the nanodiamonds can also be conjugated with biomolecular building blocks such as DNA[10] and amino acids.[11] Because of their outstanding physical properties, stable photoluminescence, and lack of photobleaching and photoblinking, nanodiamonds have been successfully applied as biomarkers or tracers.[12−18] It has been reported that fluorescein and magnetic resonance imaging contrast agents can be conjugated onto nanodiamonds to enhance imaging.[19,20]

To achieve the goal of multifunctionalization of nanodiamonds, numerous techniques for surface modification of detonation nanodiamonds to facilitate conjugation have been developed. The surface of nanodiamonds can be modified with halogens by plasma treatment or photochemical reaction.[4,21−24] Moreover, Kruger and co-workers modified nanodiamonds by exploiting a silane linker with an amino group, which could be applied to peptide synthesis and biomolecular building block conjugation.[11] It has also been reported that detonation nanodiamonds can be functionalized through diazonium coupling to complete the Suzuki coupling reaction on the nanodiamonds.[26] In general, detonation nanodiamonds have

Received: January 16, 2014
Accepted: April 25, 2014
Published: April 25, 2014

been treated with acid or ozone (O_3) to oxidize the surface of the nanodiamonds to generate carboxyl (COOH) groups.[10,11,27,28] The carboxyl group can be converted to carboxyl chloride for conjugation,[10,29] or it can be reduced by borane (BH_3) to generate the hydroxyl group (OH) for further application.[11,30,31] Recently, Mutin and co-workers reported surface phosphorylation of nanodiamonds, which enhanced their thermal stability.[32]

The thiol group (−SH) can be found in proteins, antibodies, and other biomolecules but in low numbers compared to amines or carboxyl groups. Therefore, by using the thiol group for conjugation with target molecules, the numbers and positions of the modification sites can be controlled.[33] Moreover, the thiol groups exhibit a strong affinity for gold and thus have been applied for self-assembly and gold nanoparticle (Au NP) conjugation.[21,34] One method of incorporating a thiol group onto the surface of the nanodiamond involves conjugation of a space linker with the thiol group.[35−37] Nakamura and co-workers first reported a photochemical reaction for direct modification of sulfur on the surface of the diamond powder by treatment with elemental sulfur and carbon disulfide.[38] Fokin and co-workers reported an organic reaction of diamondoids to replace the tertiary or bridgehead hydroxyl groups with thiol groups.[39,40] However, there is currently no method for the synthesis of diamondoids, which are only obtained from petroleum.[41,42] On the other hand, detonation nanodiamonds can be mass produced at low cost by detonation of explosives in a specific chamber.[4,23,32] Herein, we present a simple method for direct functionalization of detonation nanodiamonds with the thiol group by applying a modified Fokin's method to diamondoids. Compared to the photochemical reaction for thiol modification, the current method is operationally facile and can be applied to mass production. These new materials can be utilized for the conjugation of antibodies, proteins, organic molecules, and gold materials to produce a new series of nanodiamond materials.

EXPERIMENTAL SECTION

Chemicals. ND (average diameter 5 and 100 nm; Nanodiamond, TI, Switzerland) was synthesized from the detonation of a mixture of trinitrotoluene and hexogen. All chemicals were used as received without purification. Lithium aluminum hydride ($LiAlH_4$), thiourea, and hydrobromic acid were purchased from Sigma-Aldrich.

Approximately 13 nm Au NPs were prepared by citrate reduction of $HAuCl_4$. An aqueous solution of $HAuCl_4$ (1 mM, 20 mL) was refluxed at 110 °C with stirring in an oil bath. A solution of trisodium citrate (2 mL of a 38.8 mM aqueous solution) was added quickly, which resulted in a series of color changes before finally achieving a wine red solution. The mixture was refluxed for another 10 min and allowed to cool to room temperature.

Instrumentation. The Raman spectra were acquired on the micro-Raman system built in our laboratory, employing a 532 nm diode laser as the excitation source. The scattered light was filtered using the long-pass Semrock filter, and the signal was analyzed using a DK480 monochromator. High-resolution transmission electron microscopy (TEM) images were recorded using a JEOL JEM-2100F electron microscope operating at 200 kV. The UV−vis spectra were recorded on NanoDrop 2000c spectrometer (Thermo Fisher Scientific Inc.) using a quartz cuvette of 1 cm path length. The X-ray photoelectron spectroscopy (XPS) spectra were acquired on an ULVAC-PHI Quantera SXM system, using Al Kα as the X-ray source. The excitation area was 100 × 100 μm; the step sizes for survey and chemical state spectra were 1.0 and 0.050 eV, respectively. The survey and chemical state scans were performed at 280 eV pass energy with a 1 eV step and 55 eV pass energy with a 0.05 eV step. Elemental analysis (EA) data were obtained from Elementar vario EL III and Elementar vario EL cube, Elementar Analysensysteme GmbH, Germany.

RESULTS

Preparation of Carboxylated Nanodiamonds (ND-COOH). A suspension of detonation nanodiamond particles (500 mg) in a mixture of H_2SO_4 and HNO_3 (80 mL, 3/1, 60 mL/20 mL) was stirred at 100 °C for 72 h. The reaction mixture was poured into distilled water (500 mL) and separated by centrifugation at 8000 rpm. The pellets were rinsed with distilled water several times and dried in a vacuum oven at 50 °C for 24 h.

Preparation of Hydroxylated Nanodiamonds (ND-OH). ND-COOH (300 mg) and $LiAlH_4$ (113.9 mg, 3.000 mmol) were combined in dried tetrahydrofuran (15 mL). The reaction mixture was stirred under reflux for 24 h. After cooling to room temperature, the mixture was hydrolyzed with 1.0 N hydrochloric acid until no further evolution of hydrogen gas was observed. The pellets were rinsed with distilled water and acetone several times and separated by centrifugation at 8000 rpm. The resulting ND-OH was dried in a vacuum oven at 50 °C for 24 h.

Preparation of Thiolated Nanodiamonds (ND-SH). A mixture of ND-OH (200 mg) and thiourea (11.4 g, 150 mmol) in hydrobromic acid (25 mL) and glacial acetic acid (50 mL) was stirred under reflux for 48 h. The hot reaction mixture was poured in small portions into a cold (ice bath) 15% aqueous sodium hydroxide solution (600 mL) and stirred overnight at room temperature. The reaction mixture was acidified with 50% aqueous H_2SO_4 to pH = 2−3 while maintaining the temperature below 10 °C. The pellets were rinsed with distilled water several times and separated by centrifugation at 8000 rpm. ND-SH was dried in a vacuum oven at 50 °C for 24 h.

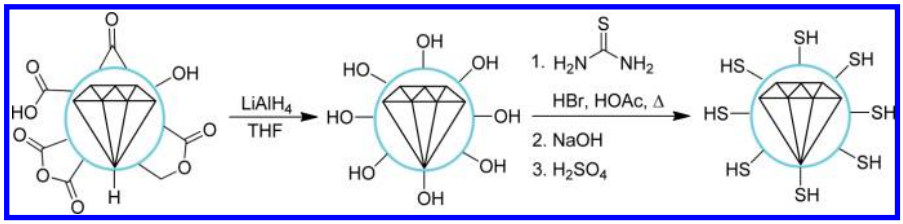
Cell Culture and Cell Viability Assay. The human lung carcinoma cell line (BCRC number: 60074) was purchased from Bioresource Collection and Research Center (BCRC, Taiwan) and had been isolated from a lung carcinoma of a 58-year-old male. A-549 cells were cultured in a 90% Ham's F12K medium with 2 mM L-glutamine and adjusted to contain 1.5 g/L sodium bicarbonate and 10% fetal bovine serum at 37 °C in a humidified atmosphere with 5% CO_2.

Powders of 5 nm ND-SH and 100 nm ND-SH were freshly suspended in sterilized distilled water followed by ultrasonication for 20 min in a biological safety cabinet prior to cell viability assay. Exponentially growing cells were seeded into a 96-well plate at 5000 cells per well for 20 h. Then the cells were treated with 0.1, 1.0, 10, and 50 μg/mL nanodiamonds for 24 h in a complete medium. After treatment, the medium was replaced by 0.5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in a complete medium at 37 °C for 3 h. Surviving cells converted MTT to insoluble blue-purple formazan, and then dimethyl sulfoxide was added to each well to dissolve formazan for 20 min. The absorbance was measured at 570 nm using a microplate ELISA reader (VersaMax, Molecular Devices). The percentage of cell viability was calculated by dividing the absorbance of treated cells by that of untreated cells.

DISCUSSION

Herein, we report a method of functionalizing the surface of detonation nanodiamonds with the thiol group, which provides a new platform for the conjugation of nanodiamonds with

Scheme 1. Direct Thiolation of the Surface of Detonation Nanodiamonds



biocompatible materials. The detonation nanodiamonds were treated with lithium aluminum hydride ($LiAlH_4$) to reduce the carboxyl and carbonyl groups on the surface to hydroxyl groups (Scheme 1). The hydroxyl groups were then replaced with thiol groups by treatment with thiourea in the presence of hydrobromic acid and acetic acid. Under acidic conditions, the hydroxyl groups were protonated; the sulfur atom of thiourea subsequently attacked and replaced the hydroxyl groups to give thiol groups (see the Supporting Information, Figure S1, for details).

The ND-SH sample was characterized by TEM, EA, Raman spectroscopy, and XPS. Raman spectroscopy is a technique to monitor exciting vibrational modes of molecules. We take advantage of this to analyze the nanodiamond. Raman spectra of ND-SH were acquired in the ranges of 1000−1800 and 2400−2800 cm^{-1} to evaluate the characteristic peaks of the thiol bond. The Raman spectrum of 5 nm ND-SH shows a major diamond signal at 1328 cm^{-1} (Figure 1) along with graphite (G band, 1580 cm^{-1}) and distorted-graphite (D band, 1328 cm^{-1}) signals. The graphite signal, G band, was contributed from the partially covered thin layer of graphite.[24,25] On the other hand, the 100 nm ND-SH spectrum showed a significantly strong diamond signal at 1329 cm^{-1}, the intensity of which obscured observation of other minor peaks (see the Supporting Information, Figure S2, for the 100 nm ND-SH detailed Raman data). The characteristic thiol group signal at 2670 cm^{-1} was observed, which demonstrated the successful introduction of the thiol group onto the surface of the nanodiamonds. The TEM images of 5 nm nanodiamonds before and after thiolation had no apparent difference of morphology (see the Supporting Information, Figure S2). EA showed a significant increase in the sulfur content after thiolation of the materials (Table 1). The percentage of sulfur was increased to 3.427% for 100 nm ND-SH and to 9.95% for 5 nm ND-SH. Compared to 100 nm ND-SH, 5 nm ND-SH had a higher surface-to-volume ratio (specific surface area), which provided more hydroxyl groups that could be replaced with thiol groups. Moreover, 100 nm ND-SH contained more carbon inside the core, which was indicated by a higher percentage of carbon in EA.

Figure 2 displays the XPS spectra of ND-SH (100 nm) that were used to evaluate the surface modification. The XPS results show the main carbon peak (C 1s) at 285 eV and the S 2s and 2p peaks at 228 and 164 eV, consistent with the report by Nakamura and co-workers[38] and Shenderova and co-workers.[43] Peak-fitting results show a band in the sulfur region at 164 eV, corresponding to the C−SH bond, that contained contributions from the bands at 164.15 (S $2p^{1/2}$) and 162.95 (S $2p^{3/2}$) eV. Moreover, a minor peak was observed in the S 2p region at 168 eV, indicative of higher binding energy of sulfur, and was attributed to the $C-SO_2$ bonds. The surface percentage of thiol groups was also estimated to be 1.07% for the surface of 100 nm ND-SH and 2.21% for 5 nm ND-SH based on XPS (see the Supporting Information, Figure S4, for the detailed 5 nm ND-SH XPS data).

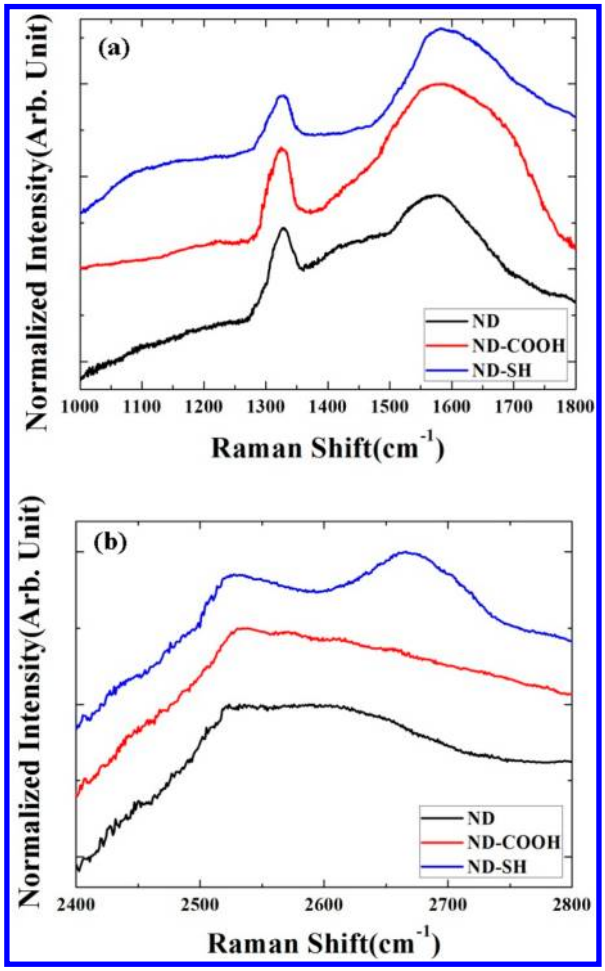


Figure 1. Raman spectra (a) from 1000 to 1800 cm^{-1} and (b) from 2400 to 2800 cm^{-1} of 5 nm ND-SH.

Table 1. Elemental Composition (%) of Functionalized Diamond Materials

	N %	C %	H %	S %
5 nm ND	2.087	85.103	1.224	0.492
5 nm ND-OH	1.774	63.249	2.418	0.394
5 nm ND-SH	0.964	40.239	1.100	9.950
100 nm ND	0.236	96.600	0.223	0.265
100 nm ND-OH	0.206	84.671	0.719	0.447
100 nm ND-SH	0.235	96.771	0.182	3.427

Thiolation of ND-SH was evaluated by treating ND-SH with Au NPs. The characteristic UV absorption of Au NPs was observed upon evaluation of ND-SH (Figure 3), which indicated that the Au NP was together with ND-SH. Another

dx.doi.org/10.1021/am500324z | ACS Appl. Mater. Interfaces 2014, 6, 7198−7203

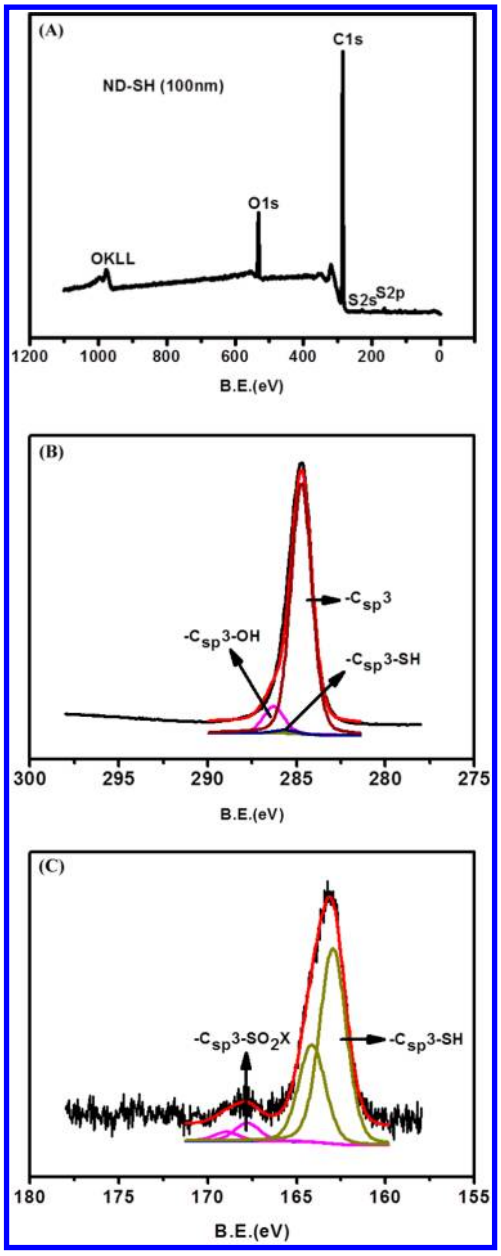


Figure 2. (A) XPS spectrum, (B) C 1s spectra, and (C) S 2p spectra of 100 nm ND-SH.

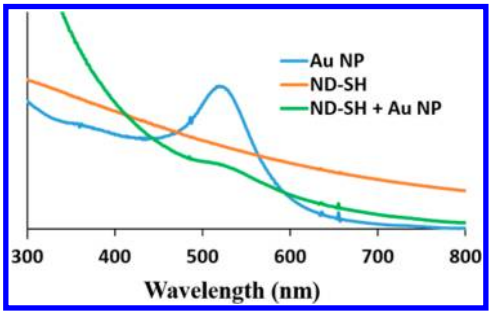


Figure 3. UV spectra of Au NPs after treatment with ND-SH, Au NPs alone, and ND-SH alone.

UV absorption experiment showed that of the Au NP peak was shifted from 525 to 528 nm when ND-SH was added to the Au NP solution (see the Supporting Information, Figure S5, for details). This phenomenon indicated that the surface plasma resonances of Au NPs were changed because of conjugation with the thiol groups to the surface of Au NPs.[34] Compared to most examples of surface conjugation of Au NPs in the literature, the surface of Au NPs was fully covered with substrates, and then an obvious red shift of UV absorption of Au NPs could be observed. Therefore, in our conditions, the shorter red shift (3 nm) of UV absorption of Au NPs was due to only part of the surface of Au NPs conjugated with the thiol groups of ND-SH. Within 30 min, the UV absorption intensity dropped to half of the original (see the Supporting Information, Figure S5, for details). Because each nanodiamond bears numerous thiol groups on the surface, the nanodiamonds can conjugate with several Au NPs to form a cross-linking bulky cluster and thus precipitate. The TEM results provide solid evidence that the thiol groups of ND-SH were conjugated with Au NPs (Figure 4). Currently, multifunctional nanocomplexes

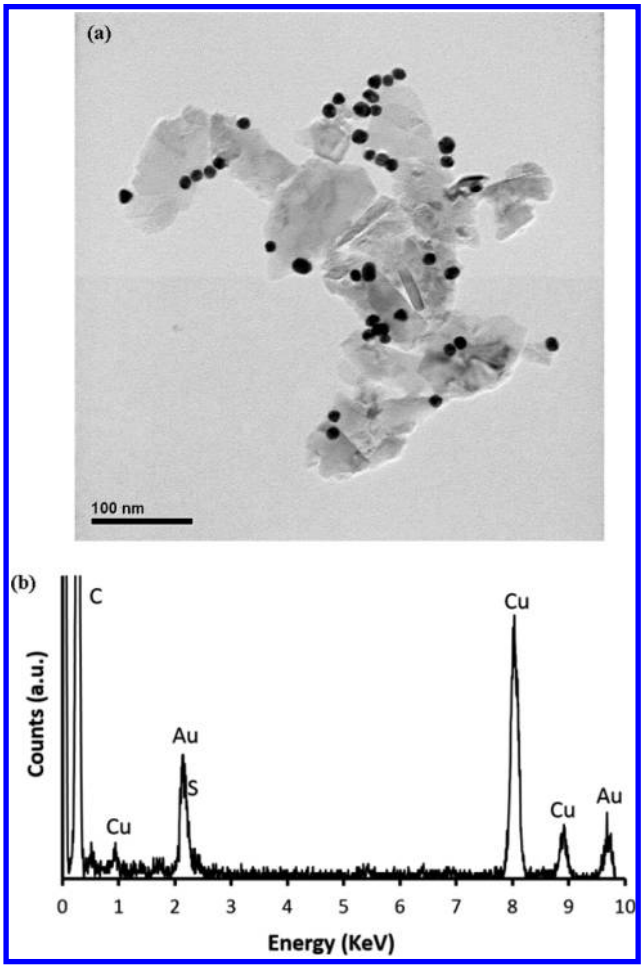


Figure 4. (a) TEM image of ND-SH conjugated with Au NPs. (b) EDS pattern of ND-S-Au on the surface of a copper holey carbon support grid.

are being extensively developed in various biomedical applications due to their different functions and extensive thermal stability. Au NPs are the most popular nanomaterials for biomedical applications such as photothermal therapy, contrast agents of CT (computed tomography), cancer therapy, and drug carriers. Because of their low toxicity and ease of detection, gold nanomaterials have been approved for use in clinical tests.[44] In most methods of surface modification of gold

nanomaterials, thiol groups are usually selected to conjugate to the surface of Au NPs with formation of a covalent bond. In this manuscript, we demonstrated our ND-SH could be successful to conjugate with Au NPs, and these results also confirmed that ND-SH actually has thiol groups after our synthetic method.

The toxicity of detonation nanodiamonds toward various cell lines has previously been investigated, illustrating that detonation nanodiamonds were not cytotoxic. Herein, we present a new type of nanodiamond; thus, the toxicity is a serious concern for prospective applications. The viability of the human lung carcinoma cells treated with ND-SH was analyzed by MTT assay. The viabilities of cells treated by 5 nm ND-SH and 100 nm ND-SH are similar (more than 90%). There is no significant difference in the cytotoxicity between cells treated by 5 nm ND-SH and 100 nm ND-SH with various concentrations (0.1, 1.0, 10, and 50 μg/mL) of NDs in a culture medium (Figure 5). Man et al. and Liu et al. have indicated outstanding

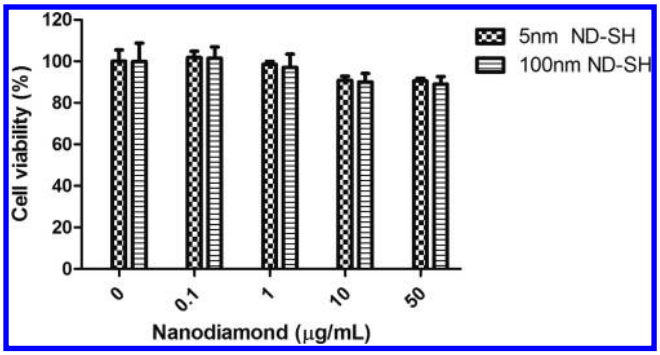


Figure 5. MTT cell viability of the human lung carcinoma cells treated with (or without) various concentrations (0.1−50 μg/mL) of 5 nm ND-SH and 100 nm ND-SH for 24 h. Results were obtained from four separate experiments; bars represent mean ± standard error of mean.

biocompatibility of pristine NDs based on more than 90% cell viability.[45,46] Lien et al. have reported the cell viability of A549 cells treated by a fluorescent and magnetic nanodiamond (FMND) particle (surface grafting with polyacrylic acids and fluorescein *o*-methacrylate), showing ~90% of viable cells incubated with 50 μg/mL NDs for 24 h.[47] In comparison with previous works of pristine NDs and surface-modified NDs, the directly thiolated NDs in our present work do not show significant cytotoxicity, which makes them promising platforms for nanomedicine.

CONCLUSIONS

In conclusion, this study presents a simple and easy approach for modification of the surface of detonation nanodiamonds with thiol groups (−SH). ND-SH possess numerous surface thiol groups that facilitate reaction with Au NPs or can be easily conjugated with functional groups with thiol affinity. The material was characterized by various spectroscopic techniques, including UV−vis, EA, TEM, XPS, and Raman spectroscopy, demonstrating successful modification of the surface of NDs with thiol groups. The atomic percentage of sulfur was 3.427% for 100 nm ND-SH and 9.95% for 5 nm ND-SH, as determined by EA. The surface percentage of thiol groups was 1.07% for 100 nm ND-SH and 2.21% for 5 nm ND-SH, as estimated from XPS. This material did not show significant toxicity to the human lung carcinoma cell line and may prospectively be applied in the biological field. This study may significantly improve the approach toward surface modification of detonation nanodiamonds and provide a new platform for the application of nanodiamonds.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in the text, mechanism of thiolation, Raman spectra of 100 nm ND-SH, XPS spectra of 5 nm ND-SH, TEM image of 5 nm nanodiamonds, and UV−vis spectra of Au NPs in the presence of ND-SH. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: +886-3-573-1180. Fax: +886-3-572-5974. E-mail: mhhsu@mx.nthu.edu.tw.

Author Contributions

M.-H.H. was the principle investigator, who conceived the project and developed the concept on which it is based. H.C. performed the ND-SH experiments and prepared the manuscript. F.-Y.C. and C.-C.C. performed the TEM experiments and discussed the results. Y.-P.H. and C.-C.H. performed the XPS experiments and discussed the results. J.-Y.C. designed the cell viability experiments and prepared the manuscript. S.-C.H. performed the cell viability experiments. J.-K.C. performed the cell viability experiments and discussed the results. D.-S.W. and H.-L.C. performed the Raman experiments.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

For financial support, we thank the National Tsing Hua University, National Health Research Institutes (Grant NM-102-PP-07), and the Ministry of Science and Technology, Republic of China. We gratefully acknowledge the support of elemental analysis by Prof. Ken-Tsung Wong and Ching-Wei Lu in the Precision Instrumentation Center, National Taiwan University.

REFERENCES

(1) Kateb, B.; Chiu, K.; Black, K. L.; Yamamoto, V.; Khalsa, B.; Ljubimova, J. Y.; Ding, H.; Patil, R.; Portilla-Arias, J. A.; Modo, M.; Moore, D. F.; Farahani, K.; Okun, M. S.; Prakash, N.; Neman, J.; Ahdoot, D.; Grundfest, W.; Nikzad, S.; Heiss, J. D. Nanoplatforms for Constructing New Approaches to Cancer Treatment, Imaging, and Drug Delivery: What should be the Policy? *NeuroImage* **2011**, *54*, 106−124.

(2) Enoki, T.; Takai, K.; Osipov, V.; Baidakova, M.; Vul, A. Nanographene and Nanodiamond; New Members in the Nanocarbon Family. *Chem.—Asian J.* **2009**, *4*, 796−804.

(3) Danilenko, V. V. On the History of the Discovery of Nanodiamond Synthesis. *Phys. Solid State* **2004**, *46*, 595−599.

(4) Krueger, A. New Carbon Materials: Biological Applications of Functionalized Nanodiamond Materials. *Chem.—Eur. J.* **2008**, *14*, 1382−1390.

(5) Xing, Y.; Dai, L. Nanodiamonds for Nanomedicine. *Nanomedicine* **2009**, *4*, 207−218.

(6) Huang, H.; Pierstoff, E.; Osawa, E.; Ho, D. Active Nanodiamond Hydrogels for Chemotherapeutic Delivery. *Nano Lett.* **2007**, *7*, 3305−3314.

(7) Lam, R.; Chen, M.; Pierstorff, E.; Huang, H.; Osawa, E.; Ho, D. Nanodiamond-embedded Microfilm for Localized Chemotherapeutic Elution. *ACS Nano* **2008**, *2*, 2095−2102.

(8) Chen, M.; Pierstorff, E. D.; Lam, R.; Li, S. Y.; Huang, H.; Osawa, E.; Ho, D. Nanodiamond-mediated Delivery of Water-insoluble Therapeutics. *ACS Nano* **2009**, *3*, 2016−2022.
(9) Zhu, Y.; Li, J.; Li, W.; Zhang, Y.; Yang, X.; Chen, N.; Sun, Y.; Zhao, Y.; Fan, C.; Huang, Q. The Biocompatibility of Nanodiamonds and Their Application in Drug Delivery Systems. *Theranostics* **2012**, *2*, 302−312.
(10) Ushizawa, K.; Sato, Y.; Mitsumori, T.; Machinami, T.; Ueda, T.; Ando, T. Covalent Immobilization of DNA on Diamond and its Verification by Diffuse Reflectance Infrared Spectroscopy. *Chem. Phys. Lett.* **2002**, *351*, 105−108.
(11) Kruger, A.; Liang, Y.; Gerald, J.; Stegk, J. Surface Functionalization of Detonation Diamond Suitable for Biological Applications. *J. Mater. Chem.* **2006**, *16*, 2322−2328.
(12) Fu, C. C.; Lee, H. Y.; Chen, K.; Lim, T. S.; Wu, H. Y.; Lin, P. K.; Tsao, P. H.; Chang, H. C.; Fann, W. Characterization and Application of Single Fluorescent Nanodiamonds as Cellular Biomarkers. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 727−732.
(13) Liu, K. K.; Cheng, C. L.; Chang, C. C.; Chao, J. I. Biocompatible and Detectable Carboxylated Nanodiamond on Human Cell. *Nanotechnology* **2007**, *18*, 325102.
(14) Chang, Y. P.; Pinaud, F.; Antelman, J.; Weiss, S. Tracking Biomolecules in Live Cells Using Quantum Dots. *J. Biophoton.* **2008**, *1*, 287−298.
(15) Chang, Y. R.; Lee, H. Y.; Chen, K.; Chang, C. C.; Tsai, D. S.; Fu, C. C.; Lim, T. S.; Tzeng, Y. K.; Fang, C. Y.; Han, C. C.; Chang, H. C.; Fann, W. Mass Production and Dynamic Imaging of Fluorescent Nanodiamonds. *Nat. Nanotechnol.* **2008**, *3*, 284−288.
(16) Liu, K. K.; Wang, C. C.; Cheng, C. L.; Chao, J. I. Endocytic Carboxylated Nanodiamond for the Labeling and Tracking of Cell Division and Differentiation in Cancer and Stem Cells. *Biomaterials* **2009**, *30*, 4249−4259.
(17) Vaijayanthimala, V.; Chang, H. C. Functionalized Fluorescent Nanodiamonds for Biomedical Applications. *Nanomedicine* **2009**, *4*, 47−55.
(18) Hui, Y. Y.; Cheng, C. L.; Chang, H. C. Nanodiamonds for Optical Bioimaging. *J. Phys. D: Appl. Phys.* **2010**, *43*, 374021−374032.
(19) Chang, I. P.; Hwang, K. C.; Chiang, C. S. Preparation of Fluorescent Magnetic Nanodiamonds and Cellular Imaging. *J. Am. Chem. Soc.* **2008**, *130*, 15476−15481.
(20) Manus, L. M.; Mastarone, D. J.; Waters, E. A.; Zhang, X. Q.; Schultz-Sikma, E. A.; MacRenaris, K. W.; Ho, D.; Meade, T. J. Gd(III)-Nanodiamond Conjugates for MRI Contrast Enhancement. *Nano Lett.* **2010**, *10*, 484−489.
(21) Liu, Y.; Gu, Z.; Margrave, J. L.; Khabashesku, V. N. Functionalization of Nanoscale Diamond Powder: Fluoro-, Alkyl-, Amino-, and Amino Acid-nanodiamond Derivatives. *Chem. Mater.* **2004**, *16*, 3924−3930.
(22) Krueger, A.; Lang, D. Functionality is Key: Recent Progress in the Surface Modification of Nanodiamond. *Adv. Funct. Mater.* **2012**, *22*, 890−906.
(23) Krueger, A. Diamond Nanoparticles: Jewels for Chemistry and Physics. *Adv. Mater.* **2008**, *20*, 2445−2449.
(24) Mochalin, V. N.; Shenderova, O.; Ho, D.; Gogotsi, Y. The Properties and Applications of Nanodiamonds. *Nat. Nanotechnol.* **2012**, *7*, 11−23.
(25) Chang, C.-C.; Chen, P.-H.; Chu, H.-L.; Lee, T.-C.; Chou, C.-C.; Chao, J.-I.; Su, C.-Y.; Chen, J. S.; Tsai, J.-S.; Tsai, C.-M.; Ho, Y.-P.; Sun, K. W.; Cheng, C.-L.; Chen, F.-R. Laser Induced Popcornlike Conformational Transition of Nanodiamond as a Nanoknife. *Appl. Phys. Lett.* **2008**, *93*, 033905−1−033905−3.
(26) Yeap, W. S.; Chen, S.; Loh, K. P. Detonation Nanodiamond: an Organic Platform for the Suzuki coupling of Organic Molecules. *Langmuir* **2009**, *25*, 185−191.
(27) Chang, I. P.; Hwang, K. C.; Ho, J. A.; Lin, C. C.; Hwu, R. J. R.; Horng, J. C. Facile Surface Functionalization of Nanodiamonds. *Langmuir* **2010**, *26*, 3685−3689.
(28) Shenderova, O. A.; Zhirnov, V. V.; Brenner, D. W. Carbon Nanostructure. *CRC Crit. Rev. Solid State Mater. Sci.* **2002**, *27*, 227−356.
(29) Zhang, Q.; Naito, K.; Yoshihisa, T.; Kagawa, Y. Grafting Polyimides from Nanodiamonds. *Macromolecules* **2008**, *41*, 536−538.
(30) DiMare, M. Ab Initio Computational Examination of Carbonyl Reduction by Borane: the Importance of Lewis Acid−base Interactions. *J. Org. Chem.* **1996**, *61*, 8378−8385.
(31) Huynh, V. T.; Pearson, S.; Noy, J. M.; Abboud, A.; Utama, R. H.; Lu, H.; Stenzel, M. Nanodiamonds with Surface Grafted Polymer Chains as Vehicles for Cell Imaging and Cisplatin Delivery: Enhancement of Cell Toxicity by POEGMEMA Coating. *ACS Macro Lett.* **2013**, *2*, 246−250.
(32) Presti, C.; Alauzun, J. G.; Laurencin, D.; Mutin, P. H. Improvement of the Oxidative Stability of Nanodiamonds by Surface Phosphorylation. *Chem. Mater.* **2013**, *25*, 2051−2055.
(33) Hermanson, G. T. *Bioconjugate Techniques*, 1st ed.; Academic Press: San Diego, CA, 1996; pp 56−57.
(34) Hsu, M. H.; Josephrajan, T.; Yeh, C. S.; Shieh, D. B.; Su, W. C.; Hwu, J. R. Novel Arylhydrazine-conjugated Gold Nanoparticles with DNA-cleaving Ability: the First DNA-nicking Nanomaterial. *Bioconjugate Chem.* **2007**, *18*, 1709−1712.
(35) Liang, Y.; Meinhardt, T.; Jarre, G.; Ozawa, M.; Vrdoljak, P.; Scholl, A.; Reinert, F.; Krueger, A. Deagglomeration and Surface Modification of Thermally Annealed Nanoscale Diamond. *J. Colloid Interface Sci.* **2011**, *354*, 23−30.
(36) Lud, S. Q.; Neppl, S.; Richter, G.; Bruno, P.; Gruen, D. M.; Jordan, R.; Feulner, P.; Stutzmann, M.; Garrido, J. A. Controlling Surface Functionality Through Generation of Thiol Groups in a Self-assembled Monolayer. *Langmuir* **2010**, *26*, 15895−15900.
(37) Meziane, D.; Barras, A.; Kromka, A.; Houdkova, J.; Boukherroub, R.; Szunerits, S. Thiol-yne Reaction on Boron-doped Diamond Electrodes: Application for the Electrochemical Detection of DNA−DNA Hybridization Events. *Anal. Chem.* **2012**, *84*, 194−200.
(38) Nakamura, T.; Ohana, T.; Hagiwara, Y.; Tsubota, T. Photochemical Modification of Diamond Powders with Elemental Sulfur and Their Surface-attachment Behavior on the Gold Surface. *Phys. Chem. Chem. Phys.* **2009**, *11*, 730−734.
(39) Fokin, A. A.; Schreiner, P. R.; Fokina, N. A.; Tkachenko, B. A.; Hausmann, H.; Serafin, M.; Dahl, J. E. P.; Liu, S.; Carlson, R. M. K. Reactivity of [1(2,3)4]Pentamantance (T_d-pentamantance): a Nanoscale Model of Diamond. *J. Org. Chem.* **2006**, *71*, 8532−8540.
(40) Tkachenko, B. A.; Fokina, N. A.; Chernish, L. V.; Dahl, J. E. P.; Liu, S.; Carlson, R. M. K.; Fokin, A. A.; Schreiner, P. R. Functionalized Nanodiamond Part 3: Thiolation of Tertiary/Bridgehead Alcohols. *Org. Lett.* **2006**, *8*, 1767−1770.
(41) Hegyi, A. Nanodiamond Imaging: a New Molecular Imaging Approach. Ph.D. Thesis, University of California, Berkeley, CA, 2013.
(42) Schwertfeger, H.; Fokin, A. A.; Schreiner, P. R. Diamonds are a Chemist's Best Friend: Diamondoid Chemistry Beyond Adamantane. *Angew. Chem., Int. Ed.* **2008**, *47*, 1022−1036.
(43) Shenderova, O.; Panich, A. M.; Hens, S. C.; Kuznetsov, V.; Vieth, H.-M. Hydroxylated Detonation Nanodiamond: FTIR, XPS, and NMR Studies. *J. Phys. Chem. C* **2011**, *115*, 1900−19011.
(44) Giljohann, D. A.; Seferos, D. S.; Daniel, W. L.; Massich, M. D.; Patel, P. C.; Mirkin, C. A. Gold Nanoparticles for Biology and Medicine. *Angew. Chem., Int. Ed.* **2010**, *49*, 3280−3294.
(45) Man, H. B.; Kim, H.; Kim, H. J.; Robinson, E.; Liu, W. K.; Chow, E. K.; Ho, D. Synthesis of Nanodiamond-daunorubicin Conjugates to Overcome Multidrug Chemoresistance in Leukemia. *Nanomedicine* **2014**, *10*, 359−369.
(46) Liu, K. K.; Zheng, W. W.; Wang, C. C.; Chiu, Y. C.; Cheng, C. L.; Lo, Y. S.; Chen, C.; Chao, J. I. Covalent Linkage of Nanodiamond-paclitaxel for Drug Delivery and Cancer Therapy. *Nanotechnology* **2010**, *21*, 315106.
(47) Lien, Z. Y.; Hsu, T. C.; Liu, K. K.; Liao, W. S.; Hwang, K. C.; Chao, J. I. Cancer Cell Labeling and Tracking Using Fluorescent and Magnetic Nanodiamond. *Biomaterials* **2012**, *33*, 6172−6185.