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Fabrication of Silicon Carbide Quantum Dots via Chemical-Etching Approach and Fluorescent Imaging for Living Cells

Yuepeng Song[1,2,3*], Dongsheng Gao[2*], Hyoung Seop Kim[3*], Cuiqin Qu[4], Jie Kang[1], Yanmin Zhu[1], Ziping Liu[1], Jing Guo[1,3], Lingfeng Xu[1], Chong Soo Lee[3]

[1]Shandong Provincial Key Laboratory of Horticultural Machineries and Equipments, Mechanical and Electronic Engineering College, Shandong Agricultural University, Tai'an, China
[2]College of Horticulture Science and Engineering, Shandong Agricultural University, Tai'an, China
[3]Department of Materials Science and Engineering, Pohang University of Science and Technology, Pohang, Korea
[4]State Grid of China Technology College, Tai'an, China
Email: [*]uptonsong@163.com, [*]dsgao219@163.com, [*]hyoungseopkim@gmail.com

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Abstract

A simple chemical-etching approach is used to prepare the silicon carbide quantum dots (QDs). The raw materials of silicon carbide (SiC) with homogeneous nanoparticles fabricated via self-propagating combustion synthesis are corroded in mixture etchants of nitric and hydrofluoric acid. After sonication and chromatography in the ultra-gravity field for the etched products, aqueous solution with QDs can be obtained. The microstructure evolution of raw particles and optical properties of QDs were measured. Different organophilic groups on the surface like carboxyl, oxygroup, and hyfroxy were produced in the process of etching. Fluorescent labeling and imaging for living cells of *Aureobasidium pulluans* were investigated. The results indicated that SiC QDs were not cytotoxic and could stably label due to the conjugation between organophilic groups of QDs and specific protein of cells, it can be utilized for fluorescent imaging and tracking cells with *in vivo* and long-term-distance. Moreover, mechanism and specificity of mark were also analyzed.

Keywords

Silicon Carbide Quantum Dots (QDs); Fluorescent Imaging; Living Cells; *Aureobasidium pulluans*

[*]Corresponding authors.

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1. Introduction

Quantum dots (QDs) have attracted considerable interest over the past two decades due to their remarkable luminescent properties which are known to offer several unique advantages such as size- and composition-induced tunable emission, high quantum yield, and low photobleaching. The huge interest to QDs of recent researchers is the potential application for fluorescence microscopy allowing the functional study of various molecules that have been identified in living cells through developing new probes for tagging molecules and observing changes in their cellular concentrations and activities. Moreover, further important application prospect has been expected in the scientific fields like fixed cell imaging, bioanalytical assays, biosensors, *Ex vivo* live cell imaging, *in vivo* animal targeting, and so on [1]-[4].

However, recent research results indicated that the widely used II-VI semiconductor QDs, e.g. CdSe, CdTe, CdS, and ZnSe and III-V, e.g. InP and InAs, were found to be cytotoxic to living cells through the release of free metallic cadmium ions and arsenics, even if a protective shell ZnS or a polymer on its surface were systematically and carefully added. It is just the one of the major limiting factors for the applications of II-VI and III-V QDs in efficient living cell imaging because of their cytotoxicity strongly influencing biological cell functioning [5] [6]. Therefore, hydrophilic QDs with excellent luminescent properties of biocompatible materials without this problem become important and urgent tasks.

At present, silicon carbon (SiC) nanocrystals have been investigated by many researchers because of their excellent biocompatibility (in particular blood compatibility), low density, and high rigidity, which are potentially useful in biology and medicine as well, for example, in bio-labeling [7]-[11]. Botsoa *et al*. performed pioneering work in the field of the biology application for SiC QDs [7]. Indeed, bulk SiC shows weak emission at room temperature on account of its indirect band gap. However, the emission intensity can be significantly enhanced when the crystallite size diminishes to several or tens of nanometers. In accordance with the quantum confinement (QC) effect, strong photoluminescence (PL) of the crystallites with diameters below the Bohr radius of bulk excitons can be achieved [12].

In this paper, aqueous solution with SiC QDs was fabricated via a simple chemical-etching method. After microstructure and optical properties were investigated, the bio-application of the SiC QDs was studied.

2. Experimental Procedure

Raw materials are homogeneous SiC nanoparticles (100 - 500 nm in diameter) fabricated via self-propagating combustion synthesis (SHS), from Technical Institute of Physics, Chinese Academy of Sciences [10] [11]. Mixture of acid solution of HNO_3 and HF with a ratio of 1:3 can easily corrode SiC particles into a hollow grid-like structure because of their high surface energy. After an ultrasonic cavitation for 25 min, the aqueous solution with SiC QDs was obtained by chromatography cutting in a high-gravity field.

Micro-morphologies and microstructures of the β-SiC nanoparticles were inspected by transmission electron microscopy (TEM, JEM-2100, JEOL, Japan) and scanning electronic microscopy (SEM, HITACHIS-4300, Japan).

Aureobasidium pulluans was used for fluorescent markers and long-term-distance imaged by the present SiC QDs. The culture conditions and method were listed like: 1) the strain is stored in a fungus incubator on the slant, scraped for two loops, and inoculated in sterilized seed media under sterile conditions for reactivation in a thermostatic shaker at 28˚C and 200 rpm on a thermostatic shaker for two days; 2) the amount of the activated strain with 8% (volume fraction) to mould fermentation medium with 10% (volume fraction) of the SiC QDs solution was inoculated and cultured at 28˚C and 200 rpm on a thermostatic shaker, and 3) the solution of *Aureobasidium pulluans* cultured for 2 d and 7 d were dropped onto slides and observed under a fluorescence microscope.

3. Results and Discussion

3.1. Hollow Grid-Like Structure Formation of Raw Particles in the Corrosion Process of SiC QDs

Raw materials of SiC nanoparticles of 100 - 500 nm in diameter prepared via SHS method, as shown in **Figure 1(a)**, were corroded into the mixture acid of Nitric and Hydrofluoric. The progress of the chemical etching can be expressed by the following three steps:

Firstly,

$$SiC + 2HNO_3 + 2H_2O \rightarrow 2HNO_2 + 4OH^- + SiC^{4+} \quad (1)$$

$$4OH^- + SiC^{4+} \rightarrow SiO_2 + CO_2 + 2H_2 \quad (2)$$

In the beginning of the reaction process, due to a large amount of CO_2 and H_2 present at the same time, and release of plenty of the reaction heat, this step was a drastic action. Plastic mixing of the miscible liquids and external temperature controlling can decrease the action.

Then,

$$SiO_2 + 6HF \rightarrow H_2SiF_6 + 2H_2O \quad (3)$$

Due to the formation characteristics of material preparation, the self-propagating combustion synthesis of the rapid reaction and fast cooling can lead to the non-equilibrium crystallization conditions. So, many defects on the surface of productions (crystal lattice distortion, dislocation, grain boundary and so on) will be formed, which results in the higher surface energy and lower corrosion activation energy. Rapidly corrosion can be developed without any additional energy like an electric current as reported in literature [7]-[9]. The hollow grid-like structure of the raw materials can be easily obtained after SiC nanoparticles etched for 1 h, as show in **Figure 1(b)**.

The hollow grid-like structure plays very important role in the formation process of SiC QDs. As we know, the ultrasonic cavitation effect can generate strong shock waves and micro-jet in solution [13], which can break the hollow porous particles into smaller size of SiC nanoparticles and fall to the aqueous solution.

3.2. Aqueous Solution with SiC Quantum Dots Fabrication and the Optical Properties

After the aqueous solution has been treated by ultrasonic cavitation, size distribution of the SiC particles was inhomogeneous in the size scope of <10 nm to 200 nm. In an ultra-gravity field (at least 2000 g), centrifugation chromatography for the aqueous phase solution was used to collect the top part of the suspension; containing the uniformly dispersed SiC nanoparticles. TEM imaging and size distribution of the SiC QDs were listed in the **Figures 2(a)** and **(b)**, respectively. Yellow exhibition of suspension in the aqueous solution under daylight exciting was also displayed in **Figure 2(c)**, which has been reported in many literatures [7]-[9].

As can be seen, the SiC QD dimensions are below 4 nm, obeying the center of the distribution approximately, the average diameter of 2.5 nm, which is similar to the former results [10] [11] [14]. The microscopic structure

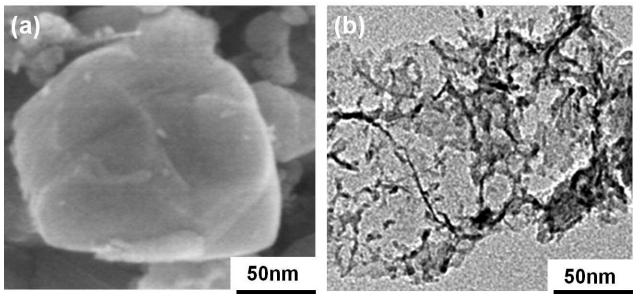
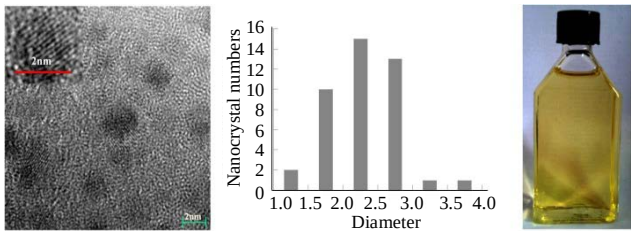


Figure 1. Morphology of SiC particles of raw (a) and after etching (b).



(a) (b) (c)

Figure 2. (a) TEM imaging; (b) Size distribution; and (c) Daylight image of 3C-SiC QD suspension in aqueous solution.

and size distribution of the QDs are the most important factors on its optical properties. Many studies verified that the core-shell spherical structure of QDs with size of less than its exciton Bohr radius will display a strong quantum confinement effect.

As the same results of the former work [10] [11] [14], the optical properties of SiC QDs can be listed as: 1) the largest value of photo luminescence (PL) will be displayed when it is excited in the scope of 320 nm to 360 nm for exciting light wavelength. The red shift for PL will occur with increasing the exciting light wavelength. 2) The PL of emission light will blue shift with the diameter decreasing of SiC QDs. There is an inherent relationship between the emission color-displaying and the sizes of the SiC QDs. Multi-color fluorescence can be displayed in one exciting wavelength according to the size. 3) The stock shift of SiC QDs will reach the value of 110 nm higher than that of fluorescence isothiocyanate (FITC) of 35 nm.

3.3. Fluorescent Labeling and Imaging for the Living Cells with SiC QDs

The *Aureobasidium pullulans* is aureobasidium of imperfect fungi and second-class fungi with the shape of yeast and hypha. Its pullulan production has excellent physicochemical and biological properties such as film-forming, fibrous, resistance oxygen, and easy decomposition [15]. Therefore, it was widely used in many fields such as medical, food packaging, and sewage treatment because of its non-toxic and harmless to human body.

The *Aureobasidium pulluan*s cells in the culture medium solution with SiC QDs for different times were dropped onto slides and observed under a fluorescence microscope with 340 nm exciting wavelengths, as shown. in **Figure 3**.

The photoluminescence effect will happen and cause strong fluorescence emission when excited by appropriate light wavelength. At the early stage of cells culturing, 3 days, for example, the *Aureobasidium pulluan*s will be stably marked on the cell membrane, displaying in the left-top position of **Figure 3(a)**. Furthermore, the SiC QD material can be absorbed and swallowed by living cells or its hypha when cultured for over 5 days (**Figure 3(b)**). Fluorescence experiments have been performed for more time after the intake of nanoparticles in the cell and the cells were found to be still living (**Figures 3(c)** and **(d)**). Compared with the control group (normal method in the cell culture medium without SiC QDs), living cells form and grow normally during each period as the same aspects reported in the literature [15]. Therefore, it is believed that the SiC QD material has no cytotoxicity on cells and does not affect functions of the producing polysaccharides. The works of Bluet *et al*. have the same conclusions [7]-[9]. This has a great significance for tracking fluorescence imaging of the living cells within long-term-distance.

Many results indicate that biological function bondings including carboxyl (COO^-), oxygroup (O^-), and hyfroxy (OH^-) on the surface can be formed after the SiC particles' etching in mixture acid of HF and HNO_3

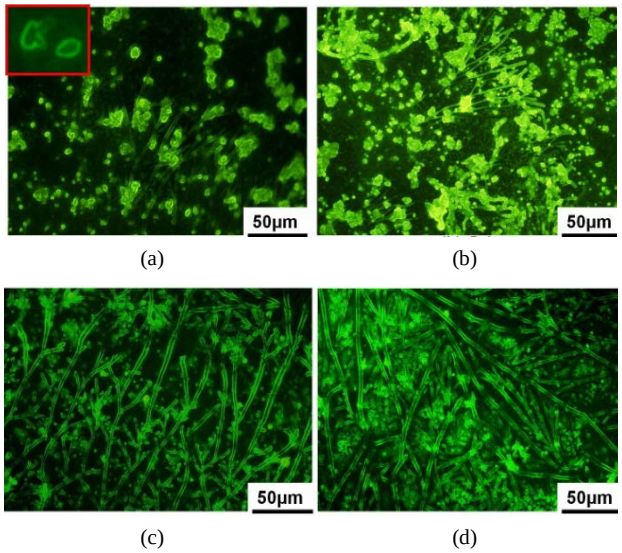


Figure 3. Fluorescence imaging of *Aureobasidium pulluans* cultured for different time. (a) 3d; (b) 5d; (c) 10d; (d) 20d.

[7]-[14]. These biological function bondings play important role in stably fluorescence labeling and imaging the living cells. In the early stage, the SiC QDs will stably label the living cells by means of coupling with biological function bondings and protein of cell membranes. As the culture time extends, SiC QDs can enter the interior of cell, marking the nucleus for example, by endocytosis. Our results are in good agreement with the former reports [10] [11].

4. Conclusion

The fabrication and application of the fluorescent SiC QDs for labeling and imaging living cells were demonstrated. The process of chemical etching, the optical properties of QDs, cell penetration, and accumulation were highlighted. This study used a homogeneous nanometer β-SiC powders which was prepared using the self-propagating combustion. After chemical etching, sonication, and chromatography in the ultra-gravity field, the aqueous solution with SiC QD products of 2.5 nm in diameter was obtained, which had a remarkable photoluminescence effect. The surface of the etched cubic β-SiC powder indicates that different organophilic groups were produced like carboxyl (COO^-), oxygroup (O^-), and hyfroxy (OH^-). The most significant among these organophilic groups may conjugate SiC QDs and specific protein. The experimental results from the treatment of *Aureobasidium pulluans* showed that the SiC QDs were not cytotoxic, and it can be utilized for long-term and long-distance fluorescent imaging of living cells. The main advantage of the elaborated SiC QDs, over conventionally used QDs based on II-VI semiconductors, is of course, their lack of cytotoxicity for *in vitro* analysis and their potential biocompatibility for *in vivo* studies.

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