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Photodynamic therapy using upconversion nanoparticles prepared by laser ablation in liquid

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ABSTRACT

Upconversion nanoparticles were prepared by laser ablation in liquid, and the potential use of the nanoparticles for cancer treatment was investigated. A Nd:YAG/SHG laser (532 nm, 13 ns, 10 Hz) was used for ablation, and the cancer treatment studied was photodynamic therapy (PDT). Morphology and crystallinity of prepared nanoparticles were examined by transmission electron microscopy and X-ray diffraction. Red and green emissions resulting from near-infrared excitation were observed by a fluorescence spectrophotometer. Generation of singlet oxygen was confirmed by a photochemical method using 1,3-diphenylisobenzofuran (DPBF). In vitro experiments using cultivated cancer cells were conducted to investigate PDT effects. Uptake of the photosensitizer by cancer cells and cytotoxicities of cancer cells were also examined. We conclude that the combination of PDT and highly crystalline nanoparticles, which were prepared by laser ablation in liquid, is an effective cancer treatment.

1. Introduction

Over the past few decades, intensive research has been performed on nanoparticles due to their unique physical properties, such as quantum confinement [1,2], and they have been used in various applications in the energy and biomedical fields [3–6]. Usually, nanoparticles are synthesized by solution methods using chemical reactions. However, these methods have the following drawbacks: (1) multistep synthesis procedures are required, (2) starting materials leave residuals after synthesis, (3) at low process temperatures, crystallinity is usually low, and (4) at high process temperatures, particle sizes increase by crystal growth. Similar problems, particularly (4), also occur in gas- and solid-phase preparation methods. Laser ablation in liquid is a useful method for fabricating nanoparticles [7–9]. The laser ablation in liquid approach has been used to fabricate noble metal nanoparticles [7–16], nanoparticles of organic materials [17], ceramics [12,18,19], and semiconductors [12]. The advantage of laser ablation in liquid is a simple procedure that yields high-purity nanoparticle solutions that are suitable for biomedical use. For ceramic nanoparticles, the advantages of laser ablation in liquid include high crystallinity, which is obtained from the fragmentation of a highly crystalline target, and formation of multi-element nanoparticles, which are difficult to obtain by solution methods. Multi-element nanoparticles are useful in various research fields, including the fabrication of optical materials such as phosphors.

One attractive property of some phosphor materials is photon upconversion, wherein visible light is emitted by an excitation of near-infrared (IR) light [20–24]. Applications for upconversion nanoparticles appear to be particularly promising in the energy and biomedical fields. One such application is their use in solar cells [25]. Usually, long wavelength light beyond the IR cannot be converted to electric energy; however, IR light could be used to generate electricity by combining upconversion nanoparticles with a normal solar cell. The upconversion nanoparticles converted IR light to visible light, and then the visible light generated electricity via the solar cell in the normal way. Scattering losses, which reduce the conversion efficiency of solar cells using only visible light, are very low for nanoparticles.

Upconversion nanoparticles are also useful in biomedical application because living bodies have low absorption for light in the near-IR region; this is called the *optical window* [26]. If upconversion nanoparticles are used as a marker for bioimaging, no surgery is needed to examine the inside of a living body [27–34]. Another biomedical application is cancer treatment; for example,

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photodynamic therapy (PDT) is one of the less-invasive therapies. After the administration of a photosensitizer to cancer cells, the irradiation of the photosensitizer with visible light generates singlet oxygen ($^1O_2$) that kills cancer cells. However, large cancers and cancers deep within the body cannot be cured by this method due to low transparency to visible light. To solve these problems, a combination of upconversion nanoparticles and a photosensitizer is planned [35–40]. After the administration of upconversion nanoparticles and a photosensitizer to cancer cells, the photosensitizer is irradiated with near-IR light, which has low absorption in a living body. The nanoparticles converted the near-IR light to visible light, and the photosensitizer generates singlet oxygen. Therefore, large cancers and those deep within the body could be cured by this method. For biomedical use, particle size is important. Particles with sizes more than approximately 200 nm are accumulated in the liver [41], while those that are less than approximately 10 nm are egested through the kidneys [42]. However, particles between approximately 10 and 200 nm are likely to be accumulated in cancer cells by the effect of enhanced permeability and retention (EPR) [43]. Surface conditions of particles are also important. Due to steric effects, particles capped with polyethylene glycol (PEG) have bioaffinity and good dispersibility [44–47].

In this study, upconversion nanoparticles prepared by laser ablation in liquid were applied to cancer treatment by PDT. Generation of singlet oxygen was examined under the excitation of near-IR light because cancer cells were killed by singlet oxygen in PDT. Furthermore, the viabilities of cancer cells by PDT were investigated using in vitro experiments. These two experiments enable us to assess the potentialities for using upconversion nanoparticles and irradiation with near-IR light in PDT.

2. Experimental

First, upconversion nanoparticles Y_2O_3:Er,Yb were prepared by laser ablation in liquid. Second, we examined the generation of singlet oxygen by the combination of upconversion nanoparticles and the photosensitizer chlorin e6. Third, in vitro experiments to study the PDT effects of this combination were performed using cultivated lung cancer cells, A549.

Before performing laser ablation in liquid, an upconversion material was synthesized by the normal precipitation method. Yttrium nitrate $Y(NO_3)_3{\cdot}6H_2O$ (4.260 g, 89.0 mol%), erbium nitrate $Er(NO_3)_3{\cdot}5H_2O$ (0.0554 g, 1.0 mol%), and ytterbium nitrate $Yb(NO_3)_3{\cdot}3H_2O$ (0.5614 g, 10.0 mol%) were dissolved in DI water (50 ml). A sodium carbonate solution (0.3 M, 50 ml) was added to this solution (0.25 M) and stirred for 2 h to obtain the precursor $Y(OH)_3$:Er,Yb as a sediment. The sediment was washed with DI water and separated by centrifugation (3000 rpm) three times. To obtain a powder of the precursor, it was dried in vacuum at 60 °C for 12 h. To obtain a powder of the upconversion material, the precursor was sintered for 30 min. Then, it was pressed at 200 MPa for 6 min and sintered at 1250 °C for 8 h in the atmosphere to fabricate a pellet to be used as the target for laser ablation in liquid, filling rate of which was 70%. To prepare upconversion nanoparticles, the target was irradiated with a Nd:YAG/SHG laser (wavelength 532 nm, pulse duration 13 ns, repetition rate 10 Hz, 0.53 mJ/pulse) for 30 min. After the nanoparticle-dispersed solution was dropped on an elastic carbon-and-copper grid and dried, the prepared particles were observed with transmission electron microscopy (TEM, 80 kV). A powder of the upconversion nanoparticles was subjected to X-ray diffraction (XRD). The upconversion spectrum of the nanoparticle-dispersed solution was measured by a fluorescence spectrophotometer with excitation at 980 nm (laser diode, continuous wave).

When upconversion nanoparticles are used for PDT, the generation of singlet oxygen is important because singlet oxygen kills cancer cells. A photochemical method with 1,3-diphenylisobenzofuran (DPBF) was used to detect singlet oxygen. Singlet oxygen decomposes DPBF, which has an absorption wavelength of 410 nm. Decreases in DPBF absorbance, as measured by the spectrophotometer, indicate the generation of singlet oxygen. Upconversion nanoparticles (Y_2O_3:Er,Yb, 3.5 μg/ml, 1.6 ml), the photosensitizer (chlorin e6 0.17 mM, 0.2 ml), and DPBF (0.1 mM, 0.2 ml) in solvent were irradiated with near-IR light (laser diode, wavelength 980 nm, continuous wave, 0.39 W/cm^2). The durations of near-IR irradiation were varied.

To understand the effects of PDT, cell viability of lung cancer cells, A549, containing upconversion nanoparticles (0.5 mg/ml) and the photosensitizer (5 μM) was measured under near-IR irradiation. The cancer cells (0.5×10^4 cells/100 μl) were cultivated under dark conditions in a 5%-CO_2 atmosphere incubator at 37 °C for 24 h in an RPMI-1610 medium with L-glutamine, which contained 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic-mixed stock solution (ABAM). Upconversion nanoparticles and the photosensitizer were added to cancer cells in a microtiter plate and cultivated under the same conditions for 24 h. Then, cancer cells were irradiated with near-IR light (laser diode, wavelength 980 nm, continuous wave).

After the 24-h cultivation, cell viability was measured by MTT assay, which was 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. For the MTT assay, MTT (10 μl) was added and left for 4 h. Then, 10% sodium dodecyl sulfate (SDS) was added and left for 24 h. Absorbance at 650 nm and 570 nm were measured for calculating cell viability. To identify the proper experimental conditions, the cytotoxicities of upconversion nanoparticles and photosensitizer to cancer cells were measured by MTT assay at each concentration as follows. Cancer cells (0.5×10^4 cells/100 μl) were cultivated for 24 h, and upconversion nanoparticles or photosensitizer was added. After 24-h cultivation, an MTT assay was performed to measure cell viability. To examine uptake of photosensitizer by cancer cells, cancer cells (0.5×10^4 cells/2 ml) in a petri dish (35 mm), which had been cultivated for 24 h, were cultured for 24 h after the addition of the photosensitizer. Optical microscopy was performed to observe the uptake.

3. Results and discussion

3.1. Size and structure of prepared nanoparticles

TEM images of the prepared nanoparticles are shown in Fig. 1. The primary particle size was several hundred nanometers, although some fine nanoparticles were observed. In previous studies [47–50], two kinds of nanoparticles were observed: coarse and fine nanoparticles. The coarse nanoparticles were aggregated polygonal particles with sizes of several hundred nanometers; these were similar to the ones shown in Fig. 1. This particle size is suitable for the EPR effect. Since the shapes and sizes of the primary particles were almost the same as those of the target for laser ablation in liquid, coarse nanoparticles would be formed by the fragmentation of the target, which was a ceramic sintered compact of nanoparticles. In general, the irradiation of a solid with a laser beam generates a shock wave and thermal shock. These effects would fragment the target. In contrast, the fine nanoparticles had sizes of several tens of nanometers. Since the particle sizes of fine nanoparticles were smaller than those of the primary particles, the sizes of fine particles would decrease during melting, evaporating, or atomizing.

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