10.1109/ULTSYM.2014.0411

In Vitro Study of PLGA/PFH Particles Loaded with Gold Nanoparticles as Theranostic Agents for Photoacoustic Imaging and Cancer Therapy

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***Abstract*—Phase-change contrast agents have been developed for photoacoustic (PA) imaging and therapy. They consist of a perfluorohexane (PFH) liquid and silica-coated gold nanoparticles (GNPs) in the core stabilized by a Poly Lactide-co-Glycolic acid (PLGA) polymer shell. PLGA is an approved biocompatible and biodegradable material for clinical applications. Silica-coated gold nanoparticles absorb visible and near-infrared wavelengths with excellent stability upon laser irradiation. In this study, PLGA/PFH particles loaded with GNPs were characterized using PA methods with a 375 MHz transducer. The vaporization was induced using a 532 nm pulsed laser. The cytotoxicity of these particles prior to vaporization was examined in cell culture, and the mechanical damage to the cancer cells by the vaporization of particles internalized in cells was investigated.**

Keywords*—PLGA/PFH particles; Photoacoustic imaging; Theranostic agents; Cancer therapy; Gold nanoparticles

INTRODUCTION

Photoacoustic (PA) imaging technology allows the detection of malignant tumors in organs such as the breast with high-contrast and high-resolution at relatively low cost [1]. However, for the early detection of malignant metastasis in tissue, exogenous contrast agents with strong optical absorption are desirable for improving the detection sensitivity [2]. Contrast agents have recently been synthesized to produce contrast in ultrasound (US) and PA imaging. US contrast agents are typically liquid [3] or gas microbubbles [4]. Gas bubbles or emulsions have been loaded with dye to serve as dual-modal contrast agents in PA and US imaging [5].

Phase-change contrast agents combining the advantages of liquid and gas bubbles contrast agents were first developed for ultrasound-based applications [6][7]. Some agents consist of superheated liquid perfluorocarbon and plasmonic nanoparticles or dyes capped by a surfactant [8], lipid [9], or protein [10] shell, and can be remotely triggered by a laser using a process called optical droplet vaporization (ODV) [11]. Poly(Lactide-co-glycolide) acid (PLGA), one of the most used synthetic biodegradable polymers, has many advantages over other shell materials. It is more stable under the ultrasonic insonificaition than monomolecular layers of lipids or surfactants [12], and can act as ligand for targeted imaging and a drug carrier in targeted drug delivery system [13].

We have developed particles containing a perfluorohexane (PFH) liquid and silica-coated gold nanoparticles (GNPs) in the core stabilized by PLGA shells, noted as PLGA/PFH-GNPs. The photoacoustic signals were measured from each individual PLGA/PFH-GNP particle as a function of laser energy until vaporization occurred. The mean vaporization threshold was determined according to the sizes of PLGA/PFH-GNPs particles. The cytotoxicity of the particles was examined before vaporization using a MCF7 human breast cancer cell line. The mechanical damage to the cells due to the vaporization effects was also examined.

METHODS

A. PLGA/PFH Particle Preparation

Gold nanospheres, 35 nm in diameter were synthesized [14] and coated with 20 nm thick silica [15], and solubilized into PFH [16]. PLGA particles containing PFH and silica-coated GNPs were prepared using a solvent evaporation process [17][18]. Briefly PLGA was dissolved in dichloromethane. GNPs-PFH solution was then added to the PLGA solution. The mixture was emulsified using a tip sonifier (BRANSO, USA). Next the emulsion was homogenized with a polyvinyl alcohol solution, and mixed with 2% isopropanol solution then stirred for 2 hours until the organic solvents evaporated. The remaining mixture was washed several times by centrifugation. The final product (PLGA particles loaded with PFH and GNPs) were collected and stored at 4°C for future use.

B. Photoacoustic Measurements

An acoustic microscope (SASAM, Kibero GmbH, Germany) was used for all photoacoustic measurements [19]. A 532 nm laser with a 330 ps pulse width, 4 kHz repetition rate, and a maximum energy of 580 nJ per pulse was focused to a 10 μm spot. The transducer used for this study has a

central frequency of 375 MHz. A drop of PLGA/PFH-GNPs water solution was loaded on top of a glass cover slip on the sample stage. Each particle was centered at the foci of the laser and transducer for photoacoustic measurements. The PA signal amplitudes were measured while the laser energy was gradually increased. The process was repeated until vaporization occurred or the maximum laser energy level (580 nJ) was reached. Three PLGA particle sizes (2, 5, and 10 μm in diameter) containing 35 nm GNPs were measured. An average of 10 particles at each size was examined.

C. PLGA/PFH-GNPs Particle Uptake by Cancer Cells

MCF7 cells were seeded at a concentration of 5×10^4 on a glass cover slip in a cell culture dish and incubated (37°C, 5% CO_2) for 48 hours. The fluorescence dye FITC (40 μL, 8 μg/mL) was added in the media and left for 24-hour incubation. Then DiI labeled PLGA/PFH-GNPs particles (50 μL, 50 μg/mL) were added to the dish. After 6-hour incubation, the cells were washed with PBS to completely remove loosely attached and free particles in the medium. Next, the fluorescent dye Hoechst (20 μL, 10 μg/mL) was added to the dish for 5 minutes. Finally, the cells were fixed with 4% formaldehyde for 15 minutes. After fixation, the cells were observed under a confocal laser scanning microscopy (LSM700, ZEISS, Germany).

For the cytotoxicity measurements, MCF7 cells were seeded in a 96-well plate (8000 cells per well) and left overnight in the incubator. Three concentrations of PLGA/PFH-GNPs (22, 67, 200 μg/mL) were add to the noncontrol wells and incubated for 24 hours. 50 μL of bleach was added to the positive control wells. The solution from each well was aspirated, and MTT solution (100 μL, 0.84 mg/mL) was added into each well. The plate was returned to the incubator for 45 minutes. Next, the MTT solution was aspirated, and 100μL of DMSO was added to each well. The absorbance was read at 590 nm on the 96-well plate reader. This test was repeated for three times.

D. Mechanical Damage from Vaporization of PLGA Particles on Cells

A half million MCF7 cells were seeded in a 60 mm diameter cell culture dish. PLGA/PFH-GNPs particles were added into the dish and incubated for 6 hours. After the cells were washed with PBS several times, 300 μL of 500 nM Propidium iodide (PI) solution was added to the cell culture dish. Then the cell culture dish was placed in the acoustic microscope. MCF7 cells containing PLGA/PFH-GNPs particles were irradiated with the laser to examine the vaporization effects of the PLGA/PFH-GNPs particles on single cells. All measurements were made at 37°C to simulate physiological conditions within the human body. Optical and fluorescence images were obtained before and after the vaporization to test the cell reaction and damage due to the vaporization events.

RESULTS AND DISCUSSION

Fig. 1A shows a TEM image of a single PLGA particle loaded with PFH liquid and silica-coated GNPs. The black dots are the GNPs and the gray background is the PLGA shell. Since the fluorinated silica-capped GNPs were well dispersed in the PFH liquid, most of them seem to be located in the core with the PFH liquid, others are mixed in the shell. The image shows that the GNPs are evenly distributed in the core of the PLGA particle. We estimate that there are about 1300 GNPs encapsulated in the PLGA particle.

After 6 hours incubation of MCF7 cells with the PLGA particles, the particles were passively internalized by the cells. The TEM image in Fig. 1B displays the PLGA/PFH-GNPs particle internalization inside a cytoplasmic vesicle. The black dots are the GNPs (white arrow). The white area is the hollow core of the PLGA formed during the fixation process (yellow arrow). The confocal fluorescence image in Fig. 1C shows the high uptake of PLGA particles (stained with DiI in red) by the cancer cells. The nuclei are dyed with Hoechst (in blue).

Fig. 2A shows a typical PA signal recorded from individual PLGA particle prior to the vaporization event. The peak-to-peak signal amplitudes were used for the calculation of PA signal intensity as functions of laser energy and particle size. Fig. 2B shows the PA signal as function of laser energy. Each dot in the graph is an average value of 10 measurements. At low energy level (<150 nJ), the signal amplitudes increase linearly with the laser energy. At higher energies, potentially because of damage to the GNPs and PLGA particles, the signal intensity stops increasing. The larger the particle size, the higher the PA signal produced since more GNPs were encapsulated inside the PLGA particles. The laser-induced vaporization threshold decreased with increasing particle size, and was 570, 330 and 145 nJ for 2, 5, 10 μm-sized PLGA particles, respectively.

The MTT assay is a protocol that measures cell viability by determining the metabolic reduction of yellow MTT tetrazolium salt to a water insoluble purple formazan. Stronger metabolic activity will produce a darker purple. After incubation with three concentrations of PLGA particles (22, 67, and 200 μg/mL) for 24 hours, the MTT assay showed that the cell viability is not affected by the presence of the PLGA particles (Fig. 3A).

Propidium iodide (PI) is membrane impermeant, and excluded from viable cells. If the cell membrane is damaged, PI will bind to the nucleic acids and fluoresce. PLGA particles within the cell were vaporized, resulting in bubble formation. In some cases, the bubble remained trapped within the cell, slowly expanding (Fig. 3B) over time. The bubble exerted pressure within the cell, and the cell slowly retracted (white arrows, Fig. 3B). For the majority of the time, membrane integrity was lost upon bubble formation, PI fluorescence was observed within 20 seconds (Fig. 3C). Some of the cells were eventually lifted off the substrate by the bubble.

These results suggest that vaporization inside the cancer cells can cause cell membrane integrity loss, resulting mechanical damage to the cell which further causes cell death (necrosis). The vaporization alone can serve as therapeutic purpose without drug assistance. This ability can be enhanced by adding therapeutic drug payloads into the particles and attaching cell-specific ligands on the surface of the particles. Future work will be focused on active targeting and controlled drug delivery in vitro and in vivo.

CONCLUSION

In this work, PLGA/PFH particles containing silica-coated gold nanoparticles were developed as phase-change contrast agents for photoacoustic imaging. In their liquid state, these agents generate strong photoacoustic signals which can be controlled by adjusting the laser energy and the particle physical properties. The particles vaporization via laser irradiation resulting in mechanical damage to the cells was demonstrated. These agents show potential as theranostic agents for photoacoustic imaging and cancer therapy.

ACKNOWLEDGMENT

The authors thank Elizabeth Berndl and Arthur Worthington (Ryerson University) for their technical assistance. This project was funded by the Canadian Institutes of Health Research (CCI-249368), the National Nature Science of China (Grant No. 81130025, 81227801, 81161120548), Natural Sciences and Engineering Research Council, and the Canada Research Chairs Program awarded to M. Kolios. Funding to purchase the equipment was provided by the Canada Foundation for Innovation, the Ontario Ministry of Research and Innovation, and Ryerson University.

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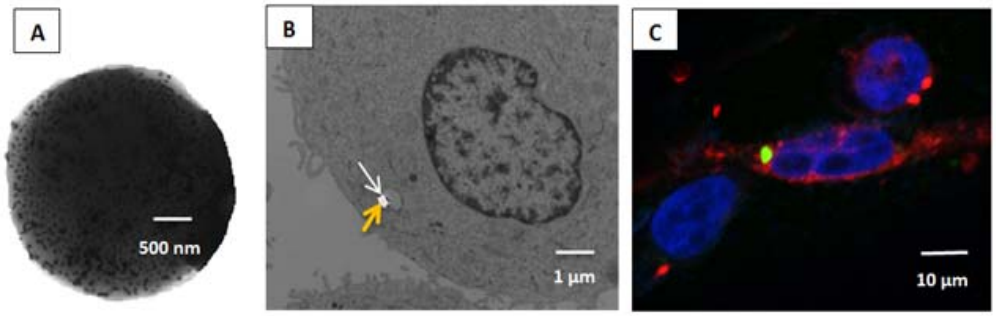

Fig. 1. (A) TEM image of a single PLGA particle loaded with PFH liquid and silica-coated GNPs. The black dots are the GNPs. The gray background is the PLGA shell. (B) TEM image of a single PLGA/PFH-GNPs particle internalized in a cytoplasmic vesicle. The black dots are the GNPs (white arrow). The white area is the hollow part of the PLGA particle (yellow arrow). (C) A confocal fluorescence image of PLGA particle uptake by MCF7 cells. The PLGA particles are labeled by DiI dye in red and are localized in the cell cytoplasm. The nuclei are stained blue by Hoechst. The cytoplasm is stained green by FITC.

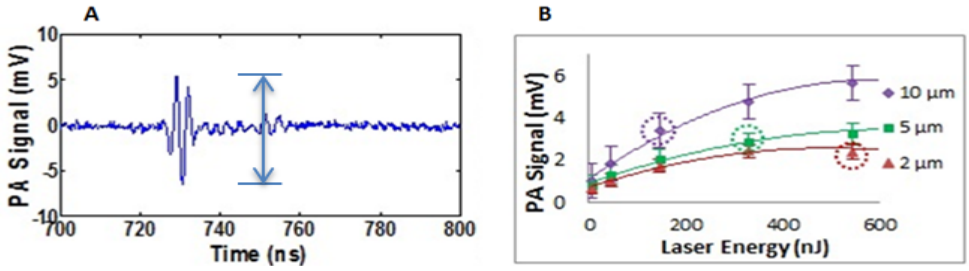

Fig. 2. (A) The photoacoustic signal obtained from a 2 µm PLGA particle. The peak-to-peak signal amplitude is used for the signal intensity comparison between different particles. (B) The PA signal as function of the laser energy for three different particle sizes. The dash circles indicate the average vaporization threshold for each size of the PLGA particles . Error bars represent the standard deviation of ten measurements on one size of the PLGA particles.

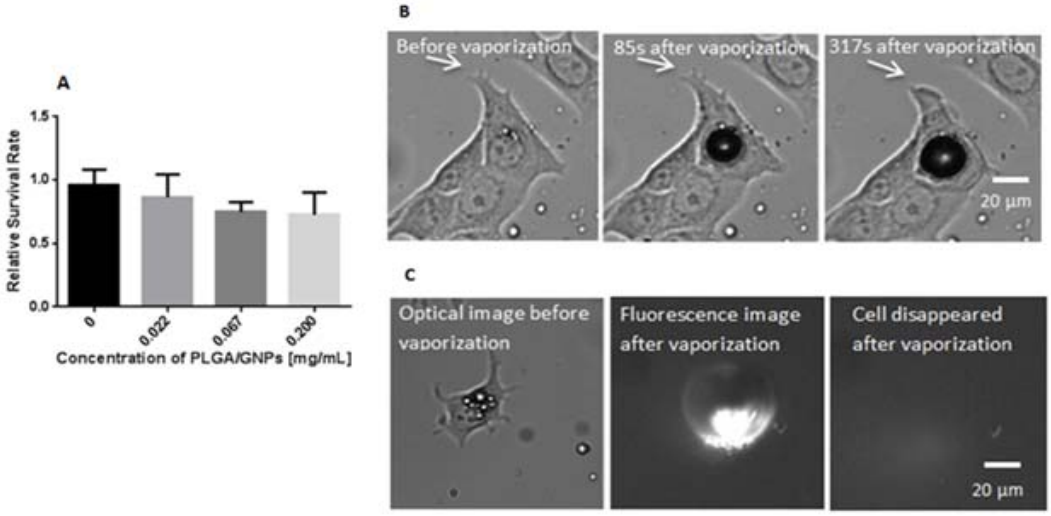

Fig. 3. (A) Cytotoxicity test using the MTT assay. The viability of cells incubated with three concentrations of PLGA particles was similar to the control group. (B) The cell slowly retracted after vaporization (indicated by the white arrows). (C) Vaporization caused membrane disintegration evidenced by the PI fluorescence 20 seconds after laser irradiation. When the cell is not attached to other cells, it was lifted off the substrate by the bubble which could be seen in the real time microscope imaging.