PLGA/PFC Particles Loaded with Gold Nanoparticles as Dual Contrast Agents for Photoacoustic and Ultrasound Imaging

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ABSTRACT

Phase-change contrast agents consisting of a perfluorocarbon (PFC) liquid core stabilized by a lipid, protein, or polymer shell have been proposed for a variety of clinical applications. Previous work has demonstrated that vaporization can be induced by laser irradiation through optical absorbers incorporated inside the droplet. In this study, Poly-lactide-co-glycolic acid (PLGA) particles loaded with PFC liquid and silica-coated gold nanoparticles (GNPs) were developed and characterized using photoacoustic (PA) methods. Microsized PLGA particles were loaded with PFC liquid and GNPs (14, 35, 55nm each with a 20nm silica shell) using a double emulsion method. The PA signal intensity and optical vaporization threshold were investigated using a 375 MHz transducer and a focused 532-nm laser (up to 450-nJ per pulse). The laser-induced vaporization threshold energy decreased with increasing GNP size. The vaporization threshold was 850, 690 and 420 mJ/cm^2 for 5μm-sized PLGA particles loaded with 14, 35 and 55 nm GNPs, respectively. The PA signal intensity increased as the laser fluence increased prior to the vaporization event. This trend was observed for all particles sizes. PLGA particles were then incubated with MDA-MB-231 breast cancer cells for 6 hours to investigate passive targeting, and the vaporization of the PLGA particles that were internalized within cells. The PLGA particles passively internalized by MDA cells were visualized via confocal fluorescence imaging. Upon PLGA particle vaporization, bubbles formed inside the cells resulting in cell destruction. This work demonstrates that GNPs-loaded PLGA/PFC particles have potential as PA theranostic agents in PA imaging and optically-triggered drug delivery systems.

Keywords: PLGA particles, Laser-induced vaporization, Theranostics, Contrast agent, Gold nanoparticles, Ultrasound, Photoacoustic

1. INTRODUCTION

Phase-change contrast agents, first developed for ultrasound-based applications[1], transform liquid emulsions into microbubble contrast agents that can have both diagnostic and therapeutic functions. When subjected to sufficient acoustic pressures delivered by an ultrasound transducer, perfluorocarbon (PFC) droplets undergo a volumetric expansion while the liquid cores change to gaseous states. This phenomenon, termed acoustic droplet vaporization (ADV)[2], has been proposed a number of in vitro and in vivo applications such as vascular occlusion[3], diagnostic imaging[4,5], ultrasound-mediated tissue ablation[6] and drug delivery[7,8]. In vitro studies have shown that the smaller the droplet diameter, the higher the vaporization threshold and high acoustic pressures are required for vaporization[9,10]. Although a nanometer-scale droplet has advantages of stability at physiological temperature and efficient for extravasation into tissue[3,8], it may require pressures higher than diagnostic ultrasound machines provide, increasing the potential for unwanted bioeffects[11].

Previous studies have shown that the vaporization of PFC droplets can be induced via optical irradiation with the facilitation of optical absorbers incorporated inside the droplets[12-15], termed optical droplet vaporization (ODV)[12,13]. When the laser fluence is below the vaporization threshold, the droplets remain in the liquid phase and can be used as a PA contrast agent. Increasing the laser energy induces droplet vaporization. The resulting microbubbles can be used for contrast enhanced ultrasound imaging. Bubble disruption can be utilized for therapeutic purposes. Optimizing laser parameters such as fluence and irradiation time, and the properties of the contrast agents, such as the type of the optical

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absorbers, type of the PFC liquids and shell materials can improve the efficiency of vaporization and further improve the degree of drug delivery.

PFC emulsions are commonly made from low-boiling point PFC liquids such as Dodecafluoropentane (PFP, 29°C) or perfluorohexane (PFH, 56°C) and stabilized by a surfactant[4,5], lipid[9,17], protein[2,10,15], or polymer[7,8,18] shell. For PA measurements, GNPs are commonly used as optical absorbing materials due to their strong absorption cross section based on the surface plasmon resonance at visible and near-infrared regions [16]. Polymer shells have many advantages over other shell materials. They are more resistant to ultrasonic waves than monomolecular layers of lipids or surfactants[20,21]; they can also act as a drug carrier and ligand for targeted imaging or targeted drug delivery[22]. Poly(L-lactide-co-glycolide) (PLGA) is one of the most used synthetic biodegradable polymers in the drug delivery system[7,8,19], and is approved for clinical application[23].

We have developed particles containing a PFH liquid and stabilized by PLGA shells, using different sizes of silica coated gold nanospheres (PLGA/PFH-GNPs). The photoacoustic signals were measured from each individual micron-sized PLGA/PFH-GNPs particle before vaporization using ultra-high frequency (375 MHz) photoacoustic measurements as a function of laser fluence. The mean vaporization threshold fluence was determined according to the sizes and types of PLGA/PFH-GNPs particles. The ODV effects in cancer cells were then examined.

2. METHODS

2.1 PLGA/PFH particle preparation

Three sizes of gold nanospheres (GNPs) (54, 75 and 95 nm) were synthesized[24], with 14, 35 and 55 nm gold cord coated with 20 nm thick silica[25], and solubilized into PFH[5,26]. PLGA particles containing PFH and silica coated GNPs with and without dialkylcarbocyanine fluorophores (DiI) dye were prepared using a double emulsion (water/oil/water) solvent evaporation process[18]. Briefly GNPs-PFH solution (0.5 mL) was added to PLGA (50 mg) in dichloromethane (1 mL). The mixture was emulsified in an ice water bath for 45 seconds, with 1-second-on, 1-second-off, 10 W pulses, using a digital sonifier (BRANSO) equipped with a microtip. Then the emulsion was homogenized with polyvinyl alcohol solution. The final emulsion was mixed with 2% isopropanol solution and stirred for 2 hours to vaporize organic solvents and washed several times by centrifugation. Deionized water (Millipore Milli-Q grade) with resistivity of 18.2 MΩ was used in all experiments. The final product of PLGA particles loaded with PFH and GNPs were collected and stored at 4°C for future use. The optical absorption spectra, particle size distribution, the morphological and structural characteristics were estimated using UV-3600 spectrophotometer, a microtrac S3500 (BETATEK INC, Canada), a scanning electron microscope (SEM) and a transmission electron microscope (TEM).

2.2 Cells uptake PLGA/PFH particles experiments

Human breast carcinoma (MDA-MB-231) cells were used for the experiments. Initially, 1×10^5 MDA cells were placed in a 60 mm in diameter cell culture dish and incubated with the fluorescent dye FITC (0.4 mg/mL) in the media for 36 hours. 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 (PH=7.4) to completely remove loosely attached and free particles in the medium. The cells were then used for PA experiments or fixed for fluorescence imaging according to the next steps. Fluorescent dye Hoechst (40 μL, 10 μg/mL) was added to the dish and fixed with 4% formaldehyde for 15 min. After fixation, the cells with DiI labeled particles were observed under a confocal laser scanning microscopy (LSM700, ZEISS, Germany).

2.3 Photoacoustic microscope

An acoustic microscope (SASAM, Kibero GmbH, Germany) was used for all photoacoustic measurements. The microscope consists of three main components: an optical microscope (IX81 Olympus, Japan) with its objectives fixed under the sample stage, a transducer positioned above the sample stage, and a focused laser (Teem Photonics, France) collimated through the side port onto the sample. A 532 nm laser was used which had a 330 ps pulse width, 4 kHz repetition rate, a 10 μm focusing spot size, and a maximum energy of 450-nJ per pulse. The transducer used for this study has a central frequency of 375 MHz , 42% bandwidth, and 60° aperture. Signals were amplified by a 40 dB amplifier (Miteq, USA) and digitized at 8 GHz (DC252, Agilent, USA).

2.4 Photoacoustic measurements

The PLGA/PFH-GNPs were diluted with water and loaded on top of a glass cover slip on the sample stage. Each droplet was centered and aligned with the laser and transducer for photoacoustic measurements. The laser fluence was increased gradually from 40 to 920 mJ/cm^2, while the PA signal amplitudes were measured for each setting. The process was repeated until vaporization occurred or the maximum laser fluence level was reached. The peak-to-peak photoacoustic signal amplitudes and vaporization thresholds as a function of laser fluence were recorded. Three types of PLGA/PFH-GNPs particles were examined, 5 μm in diameter, each containing 14 nm, 35 nm or 55 nm (in diameter) gold core nanoparticles enclosed in a 20 nm silica shell. An average of 10 particles of each type was examined. MDA cells containing PLGA/PFH-GNPs particles were irradiated to examine the vaporization effects of the particles on single cells. All measurements were made at 37°C to simulate physiological conditions within the human body.

3. RESULTS AND DISCUSSION

3.1 PLGA/PFH-GNPs particles

In this study, gold nanoshperes were selected as the optical absorbing material due to their high absorption coefficient around 532 nm, ease of synthesis, and small size permitting high-yield incorporation into micron-scale PLGA particles. A 20 nm silica coating was chosen to maximize the PA signal strength and the stability of gold nanoshperes under laser irradiation[27]. The silica shell also facilitated the GNPs misibilized into the hydrophobic and lipophobic PFH liquid[20] using the ligand exchange technique[28]. Three sizes of GNPs (14 nm, 35 nm, and 55 nm in diameter) with 20 nm silica shell each were used for the synthesis of the PLGA/PFH-GNPs particles. TEM images in Figure 1 A-C demonstrate good monodispersity of silica-coated GNPs with three sizes (13.9±2.4, 34.9±2.5 and 54.5±4.2nm in diameter of gold cores, and 20.0±5.0 nm thick silica shells) in aqueous solutions. PFH was chosen due to its historical usage, low boiling temperature (56°C), and ease of handling.

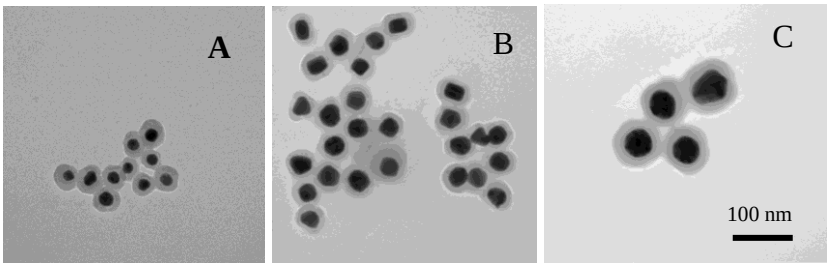

Figure 1. TEM images of silica coated GNPs, 14 nm (A), 35 nm (B), and 55 nm (C) in diameter of gold core with 20 nm silica shells. The scale bar for all figures is 100 nm.

The silica coated GNPs were suspended in the PFH liquid within the core of the PLGA particles. Figure 2A is the SEM image of PLGA/PFH-GNPs (PLGA shell thickness is approximately 10% of the particle diameter). The particle size range is from 0.5 to 12 μm (shown in Figure 2B).

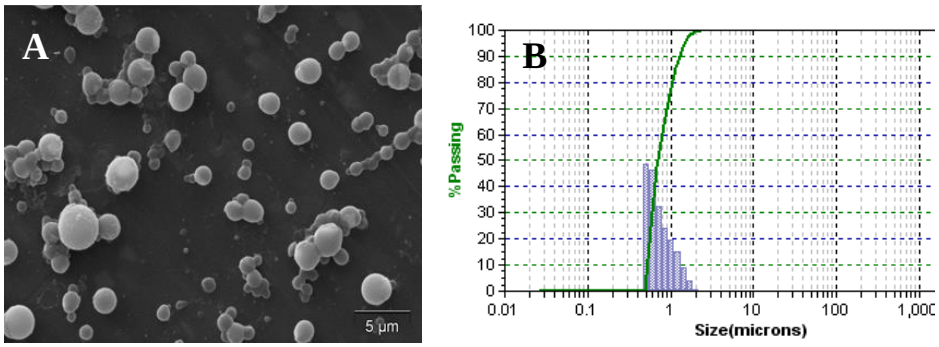

Figure 2 SEM image of PLGA/PFH-GNPs particles (A), and the particle size distribution (B).

3.2 Photoacoustic measurements

The photoacoustic signal amplitudes from single PLGA/PFH-GNPs particles were measured using a 375 MHz transducer, over a laser fluence range of 40-920 mJ/cm^2 using an approximately 200 mJ/cm^2 step size. The signal was measured from the central regions of each particle. Ten PLGA/PFH-GNPs particles were measured from each type of particle. Figure 3 shows the average PA signal amplitudes as functions of laser fluence. The signal amplitudes increase as the laser fluence increases. Also, the larger the GNPs, the higher the signal amplitudes were produced. At low laser fluence level (< 400 mJ/cm^2), the PA signal is approximately linearly proportional to the laser fluence. At higher laser fluence, non-linearity was observed. This is possibly due to deformation of PLGA shells and GNPs of PLGA/PFH-GNPs.

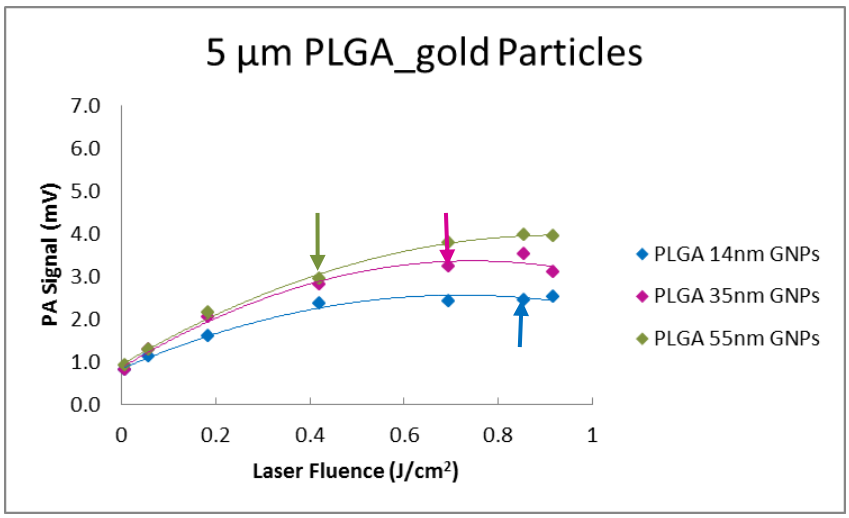

Figure 3. Photoacoustic signal amplitudes and vaporization threshold as functions of laser fluence and GNP sizes. The arrow positions indicate when more than 50% of the particles vaporized (arrows color coded to particle size).

Droplet vaporization was observed and recorded as video sequences. Figure 4 shows a vaporization process in which the bubble produced after vaporization expanded to approximately 5x the original particle size immediately after vaporization (Figure 4A and 4B). Then the bubble diameter slowly increased over time (Figure 4C). Ten particles were measured for each type of PLGA/PFH-GNPs particles. If more than 50% of particles were vaporized, then the fluence level was recorded as vaporization threshold as shown in Figure 3. The arrows indicate the position of the vaporization threshold for each type of PLGA/PFH-GNPs particle, which was 850, 690 and 420 mJ/cm^2 for 5µm PLGA/PFH-GNPs particles loaded with 14, 35, and 55nm GNPs with 20 nm silica shells, respectively. The larger size was required a lower fluence level due to the lager absorption cross section of GNPs. The fluence rates are rather high due to the low concentration of GNPs used. This will be optimized in the future work by adjusting the GNP concentration and shell properties.

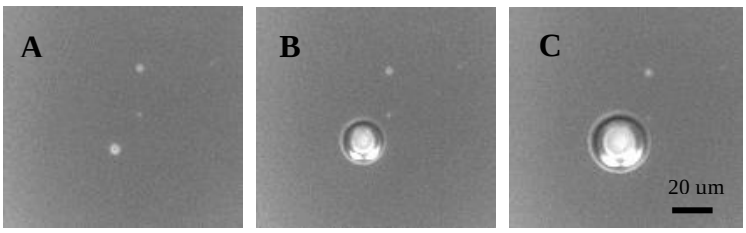

Figure 4. The optical images of PLGA/PFH-GNPs vaporization process. The initial particle diameter was 5 µm (A). A subsecond after irradiation, the bubble appeared with a size of 25 µm (B). Then bubble diameter slowly increased to 30 µm after 5 seconds (C). The scale bar is 20 µm.

3.3 Dil labeled PLGA/PFH-GNPs particles internalization in MDA cells

In Figure 5 images of MDA cells loaded with Dil-labeled PLGA/PFH-GNPs particles are shown. The images were obtained using a confocal laser scanning microscope. The cytoplasm show in green from FITC stain (Figure 5A); nuclei emit blue due to the Hoechst fluorescence (Figure 5B); and DiI labeled PLGA/PFH particles show in red fluorescence (Figure 5C). As the images indicate, a significant amount of DiI-labeled PLGA/PFH-GNPs particles were phagocytized by the MDA cells (Figure 5D). Figure 6 show the series of vaporization images of PLGA/PFH-GNPs particles inside the MDA cells. The bubble expanded, which eventually caused the mechanical damage of the cell. This result indicates the potential applications of these particles as anti-cancer therapy agents. The future work will be focused on investigation of cell death caused by vaporization.

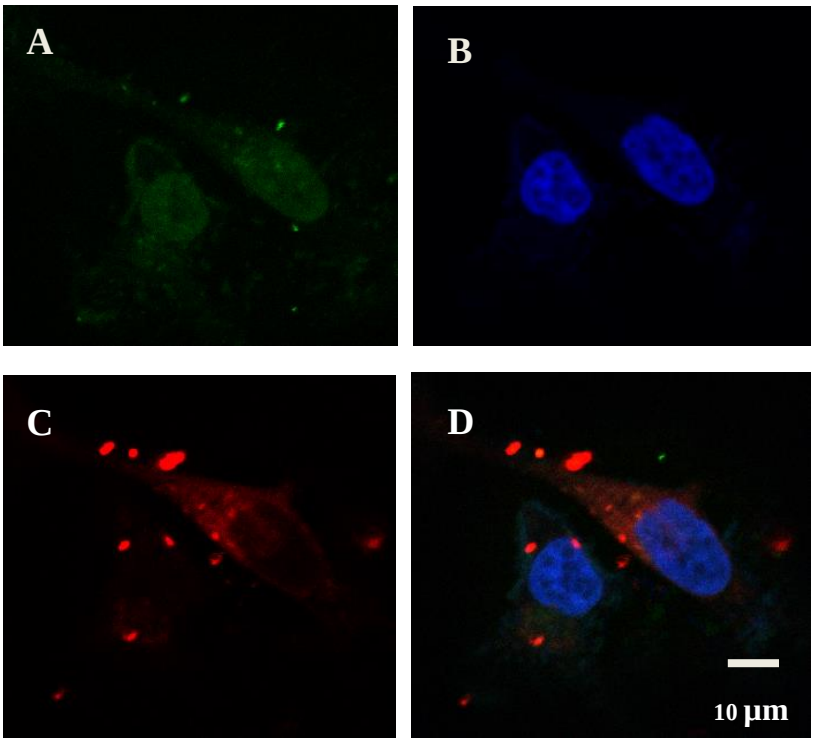

Figure 5. Confocal fluorescent images of PLGA/PFH-GNPs internalization by MDA cells. Cytoplasm stained with FITC showing in green (A). Nuclei showing in blue due to Hoechst stain. DiI labeled PLGA/PFH-GNPs particles showing in red (C). The merged image of three components is shown in D.

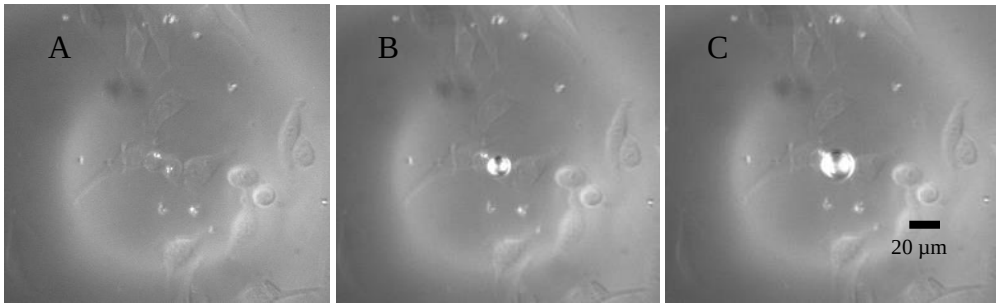

Figure 6. Optical images of vaporization sequences of PLGA/PFH-GNPs inside the cell. Time interval between sequence A and B is in subsecond range. The time elapse between B and C is 2 seconds.

4. CONCLUSION

In this study, PLGA/PFH particles containing silica coated gold nanoparticles were synthesized as phase change contrast agents for photoacoustic imaging, and their physical and optical properties were characterized using different techniques. In their liquid state, these agents generate strong photoacoustic signals which can be controlled by adjusting the laser fluence and the particle physical properties. Passive internalization by cancer cells was demonstrated, and the particles could then be vaporized via laser irradiation, resulting in mechanical damage to the cells. These agents show potential as theranostic agents for photoacoustic imaging and therapy.

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