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Vaporization, photoacoustic and acoustic characterization of PLGA/PFH particles loaded with optically absorbing materials

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***Abstract*— Poly (lactide-co-glycolic acid) (PLGA) is an FDA approved biocompatible and biodegradable material that is commonly used in implantation and drug delivery applications. It can be used as a carrier for various chemotherapeutic drugs, imaging agents and targeting moieties. Perfluorocarbon (PFC) liquids have been used in various biomedical applications, and can be activated (i.e. the liquid core converted to gas) via laser irradiation through the incorporation of optically absorbing nanoparticles or dyes within the emulsions. PLGA particles were synthesized with nanoparticles (gold or iron oxide) or dyes (DiI or rhodamine) within the PLGA shell and perfluorohexane (PFH) in the core. The photoacoustic signals and vaporization threshold of individual micron-sized particles were examined to optimize the dye and nanoparticle combination. The PLGA/PFH particles containing gold nanoparticles and DiI had the lowest vaporization threshold, with each particle consistently requiring less than 100 mJ/cm^2 for vaporization. The effects of vaporization were then tested in cell culture. The particles were internalized by MDA breast cancer cells, and then irradiated with a laser. Once the particle was vaporized, a bubble formed within the cell which destroyed the cell. This work presents a first study of using a solid-shell PLGA particle encapsulating PFH liquid as a theragnostic agent.**

Keywords—Photoacoustics, contrast agents, theragnostic agents, PLGA particles

I. INTRODUCTION

Perfluorocarbon (PFC) liquids are chemically and biologically inert, have high oxygen solubility and can be made into nano- or micron-sized emulsions for intravascular circulation. Upon acoustic irradiation of sufficient pressure, the PFC emulsion can be vaporized using a method called acoustic droplet vaporization (ADV) [1]. The gas bubbles generated using ADV can be used as ultrasound contrast agents, for cancer therapy via targeted drug delivery as well as mechanical cancer cell destruction [2]–[7].

PFC droplets can also be vaporized via optical irradiation, using a method called optical droplet vaporization (ODV) [8]. Incorporating optically absorbing nanoparticles into the PFC liquid facilitates vaporization when irradiated with sufficient laser intensity [9], [10]. This system permits the PFC emulsion to be used as theragnostic agents; at low laser energies, they can be used as photoacoustic contrast agents. Increasing the laser energy results in vaporization, which can be used for therapeutic applications. PFC emulsions are commonly made using low-boiling point PFC liquids such as perfluoropentane (PFP, 29°C) or perfluorohexane (PFH, 56°C) and stabilized by a lipid shell. For photoacoustic measurements, gold nanoparticles are commonly incorporated into the emulsion. Other optically absorbing nanoparticles or dyes are not commonly used. Various shell materials and optically absorbing agents may provide better stability and/or vaporization performance. In addition, the effects of these particles, and the vaporization mechanism have not been well studied in cell culture.

We have developed novel and stable PFC particles using Poly(lactide-co-glycolic acid) (PLGA) containing PFH, and various optically absorbing materials (DiI and rhodamine dyes, and gold or iron oxide nanoparticles). Using ultra-high frequency (UHF, over 100 MHz) photoacoustic measurements, the particles were individually examined to characterize the vaporization threshold and effects of ODV in cell culture using MDA breast cancer cells.

II. METHODS

A. PFC particle preparation

Dyes (DiI, rhodamine) and nanoparticles (gold, iron oxide) were incorporated into PLGA/PFH particles using a double emulsion (water/oil/water) evaporation process. Liposoluble dyes or nanoparticles were added to CH_3Cl dissolving PLGA agents. The mixture was emulsified using an ultrasonic probe after adding PFH in the solution. The above emulsified solution was then poured into PVA solution and homogenized within 5 min for the second emulsion. The final emulsion was mixed mechanically for 2 h with isopropanol solution to extract CH_3Cl. Subsequently, the solution was centrifuged at 3500 rpm for 3 min, the supernatant was discarded, and the precipitate

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was washed by deionized water. The process of centrifugation was repeated three times. Finally, the precipitate was collected and stored at 4°C for further use. The size distribution, the morphological and structural characterization were estimated by a scanning electron microscope (SEM) and a transmission electron microscope (TEM).

B. Photoacoustic Measurements

A SASAM acoustic microscope (Kibero GmbH, Germany) was used for all measurements. The microscope consists of an IX81 inverted optical microscope (Olympus, Japan) fitted with a transducer positioned above the sample holder. A 532 nm laser (Teem Photonics, France) was collimated through the side port and focused by a 10x optical objective onto the sample. The laser had a 330 ps pulse width, 4 kHz repetition rate and the fluence was varied between 5-100 mJ/cm^2. A 375 MHz transducer (60° aperture, 42% bandwidth) was used for acoustic pulse echo measurements, and also recorded the laser-induced photoacoustic measurements. The transducer was aligned directly over the laser spot prior to PLGA particle measurements. Signals were averaged 100 times, amplified by a 40 dB amplifier (Miteq, USA) and digitized at 8 GHz (DC252, Agilent, USA). Further details on the system can be found in [11].

The PLGA/PFH particles were diluted with water to ensure that the particles were sufficiently far apart from each other to probe them individually. The microscope stage was scanned over a 15x15 µm area using a 0.5 µm step size, with the PLGA particle located at the center. The laser fluence was increased gradually up to 400 mJ/cm^2, and the measurements repeated until vaporization occurred. The vaporization threshold of four PLGA/PFH particles were examined, each containing a dye (DiI or rhodamine) and nanoparticles (gold or iron oxide Fe_3O_4). MDA cells were incubated with PLGA/PFH particles for two hours to assess particle uptake. Cells containing PLGA/PFH particles were then irradiated to examine the vaporization effects of the particles on single cells.

III. Results and Discussion

Figure 1 shows a scanning electron microscopy image of a PLGA/PFH particle with DiI (fluorescence image inset), and a transmission electron microscopy image of a PLGA/PFH particle loaded with gold nanoparticles. The DiI-PLGA/PFH particle is about 5 µm in diameter with an indentation in the middle (figure 1A); DiI is located within the shell as indicated by the fluorescence image. The gold nanoparticles are also located around the shell, these are clearly visible as small black dots in figure 1B. In this image, the particle had been vaporized; it is likely that the expelled gas created the open flap during the vaporization process.

The optimum dye and/or nanoparticle combination was investigated by measuring the photoacoustic signal amplitude vs. laser fluence of single PLGA/PFH particles. Figure 2 shows the photoacoustic signal amplitude measured from PLGA/PFH particles containing DiI, Rhodamine, gold nanoparticles and iron oxide particles on their own. At the lowest laser fluence of 5 mJ/cm^2, the signal from particles containing DiI was stronger than the other particles. As the fluence increased, the signal decreased for particles containing DiI and rhodamine. This is attributed to photobleaching; the signal steadily decreased while the particles were continuously irradiated. The photoacoustic signal from the particles containing nanoparticles increased with increasing laser fluence. Based on this initial investigation, PLGA/PFH particles were made with gold nanoparticles and DiI (denoted DiI-AuNP PLGA/PFH), the components that gave the strongest photoacoustic signal. A dye and nanoparticle were combined so that the fluorescence could be used to identify the particles, with the nanoparticle responsible for most of the photoacoustic signal generation. Figure 2B shows a single DiI-AuNP PLGA/PFH particle before and after laser irradiation, with the bubble clearly visible after vaporization.

C-scan images of DiI-AuNP PLGA/PFH particles were made with increasing laser fluence. The c-scan image of maximum intensity, along with the a-scan from the center of the particle is shown in figure 3. As the laser fluence increased, the signal increased, with vaporization consistently occurring using less than 100 mJ/cm^2. Vaporization was confirmed by the presence of a bubble slowing expanding over time, and very strong acoustic backscatter occurring from the bubble relative to the particle prior to bubble formation. In some cases, the particle remnants could be observed at the bubble edge.

The DiI-AuNP PLGA/PFH particles were incubated with MDA cells to examine their effects in cell culture. The particles were easily internalized by the cells after two hours, which was confirmed via fluorescence and electron microscopy imaging. Particles were visible both inside and outside the cells. Upon irradiation, the particles vaporized, creating a bubble that expanded within the cell, eventually destroyed the cell (figure 4). The cells around the bubble shifted due to the pressure exerted by the expanding bubble.

IV. Conclusions

This work demonstrates PLGA/PFH particles containing optically absorbing nanoparticles for use as photoacoustic contrast agents and theragnostic agents. PLGA/PFH particles containing either gold or iron oxide nanoparticles showed high photoacoustic signal generation and vaporization efficiency. Combining the NPs with DiI enabled identification via fluorescence, and increased the photoacoustic signal while reducing the vaporization threshold. It was shown that when the particles were incorporated within cells, photoacoustic signals were detected and vaporization occurred, indicating these particles can be used as both imaging and therapy agents.

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REFERENCES

[1] O. D. Kripfgans, J. B. Fowlkes, D. L. Miller, O. P. Eldevik, and P. L. Carson, "Acoustic droplet vaporization for therapeutic and diagnostic applications," *Ultrasound in Med. & Biol.*, vol. 26, no. 7, pp. 1177–1189, 2000.

[2] S.-T. Kang and C.-K. Yeh, "Intracellular Acoustic Droplet Vaporization in a Single Peritoneal Macrophage for Drug Delivery Applications," *Langmuir*, vol. 27, no. 21, pp. 13183–13188, Nov. 2011.

[3] C.-H. Wang, S.-T. Kang, Y.-H. Lee, Y.-L. Luo, Y.-F. Huang, and C.-K. Yeh, "Aptamer-conjugated and drug-loaded acoustic droplets for ultrasound theranosis," *Biomaterials*, vol. 33, no. 6, pp. 1939–1947, Feb. 2012.

[4] M. L. Fabiilli, J. Lee, O. D. Kripfgans, P. L. Carson, and J. B. Fowlkes, "Delivery of Water-Soluble Drugs Using Acoustically Triggered Perfluorocarbon Double Emulsions," *Pharmaceutical research*, vol. 27, no. 12, pp. 2753–2765, 2010.

[5] N. Y. Rapoport, A. M. Kennedy, J. E. Shea, C. L. Scaife, and K.-H. Nam, "Controlled and targeted tumor chemotherapy by ultrasound-activated nanoemulsions/microbubbles," *Journal of Controlled Release*, vol. 138, no. 3, pp. 268–276, Sep. 2009.

[6] R. Williams, C. Wright, E. Cherin, N. Reznik, M. Lee, I. Gorelikov, F. S. Foster, N. Matsuura, and P. N. Burns, "Characterization of Submicron Phase-change Perfluorocarbon Droplets for Extravascular Ultrasound Imaging of Cancer," *Ultrasound in Medicine & Biology*, vol. 39, no. 3, pp. 475–489, Mar. 2013.

[7] P. S. Sheeran, V. P. Wong, S. Luois, R. J. McFarland, W. D. Ross, S. Feingold, T. O. Matsunaga, and P. A. Dayton, "Decafluorobutane as a Phase-Change Contrast Agent for Low-Energy Extravascular Ultrasonic Imaging," *Ultrasound in Medicine & Biology*, vol. 37, no. 9, pp. 1518–1530, Sep. 2011.

[8] E. M. Strohm, M. Rui, M. C. Kolios, I. Gorelikov, and N. Matsuura, "Optical droplet vaporization (ODV): Photoacoustic characterization of perfluorocarbon droplets," in *IEEE International Ultrasonics Symposium*, 2010, pp. 495–498.

[9] E. M. Strohm, M. Rui, I. Gorelikov, N. Matsuura, and M. Kolios, "Vaporization of perfluorocarbon droplets using optical irradiation," *Biomedical Optics Express*, vol. 2, no. 6, pp. 1432–1442, 2011.

[10] K. Wilson, K. Homan, and S. Emelianov, "Biomedical photoacoustics beyond thermal expansion using triggered nanodroplet vaporization for contrast-enhanced imaging," *Nat Commun*, vol. 3, p. 618, Jan. 2012.

[11] E. M. Strohm, I. Gorelikov, N. Matsuura, and M. C. Kolios, "Acoustic and photoacoustic characterization of micron-sized perfluorocarbon emulsions," *J. Biomed. Opt*, vol. 17, no. 9, pp. 096016–1–9, Sep. 2012.

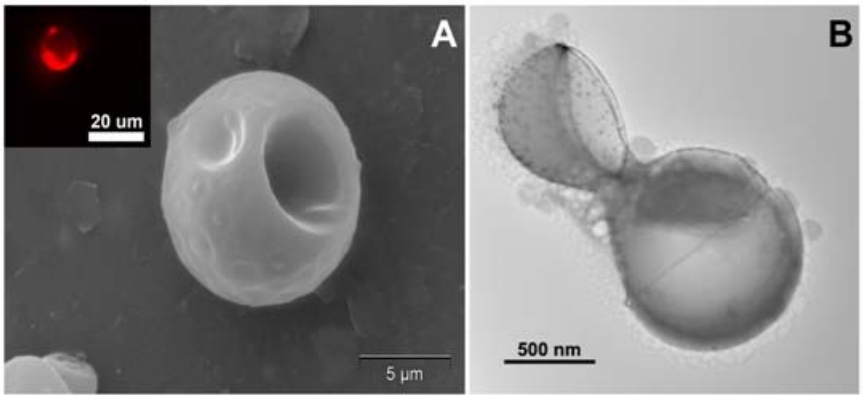

Figure 1: (A) SEM image of PLGA/PFH particles containing DiI. Fluorescence light microscope image (inset) shows DiI (red component) on the particle surface. (B) TEM image of a PLGA/PFH particle loaded with gold nanoparticles, visible as black dots located around the particle shell.

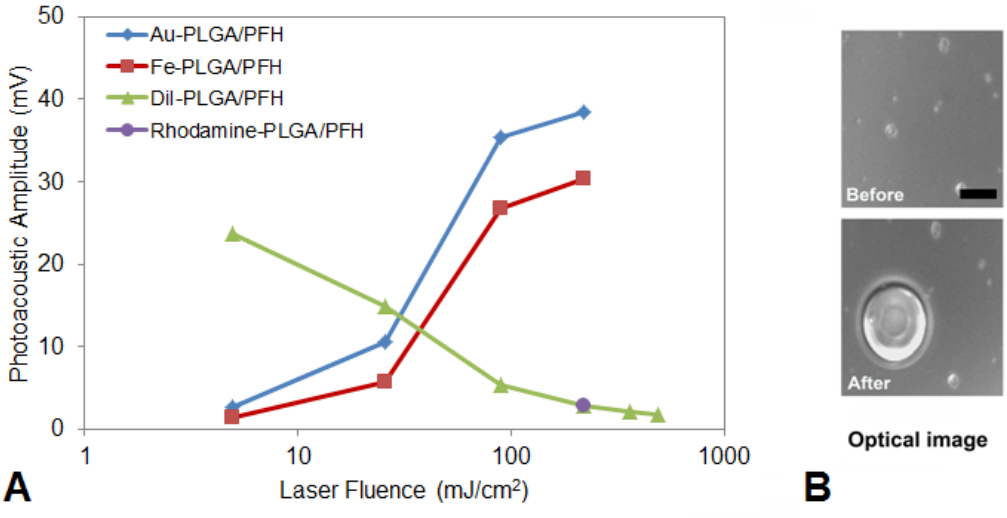

Figure 2: (A) Photoacoustic signal of different PLGA/PFH particles containing gold nanoparticles (Au), iron oxide nanoparticles (Fe), DiI or rhodamine. (B) An image of a single DiI-AuNP PLGA/PFH particle before and after laser irradiation. The scale bar is 20 µm.

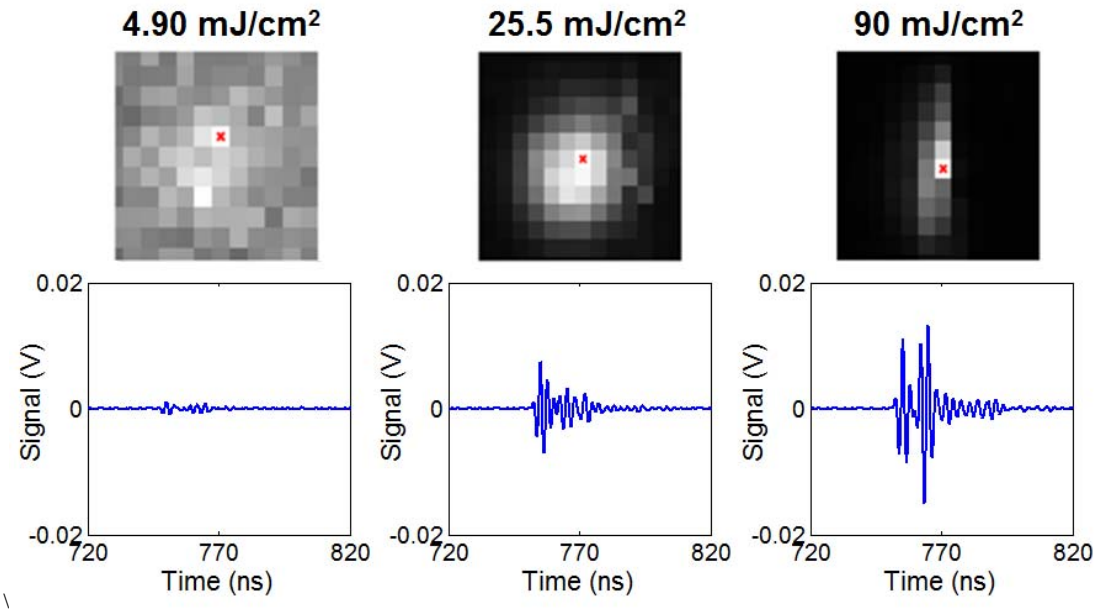

Figure 3: (Top row) C-scan photoacoustic images of a single PLGA particle with increasing laser fluence. Vaporization occurred in the last frame to the right on the red "X", as the signal was no longer detected. (Bottom row) The photoacoustic signal measured directly from the PLGA particle denoted by the red "X" in the c-scan image. The c-scan images are 12x12 μm.

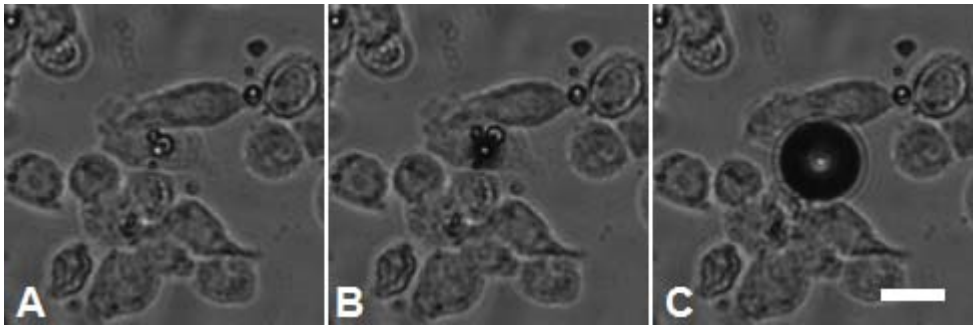

Figure 4: PLGA/PFH particles incorporated within MDA beast cancer cells before (A) and after (B, C) vaporization. The scale bar is 20 μm.