Biodegradable polymer based theranostic agents for photoacoustic imaging and cancer therapy

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ABSTRACT

In this study, multifunctional theranostic agents for photoacoustic (PA), ultrasound (US), fluorescent imaging, and for therapeutic drug delivery were developed and tested. These agents consisted of a shell made from a biodegradable Poly(lactide-co-glycolic acid) (PLGA) polymer, loaded with perfluorohexane (PFH) liquid and gold nanoparticles (GNPs) in the core, and lipophilic carbocyanines fluorescent dye DiD and therapeutic drug Paclitaxel (PAC) in the shell. Their multifunctional capacity was investigated in an *in vitro* study. The PLGA/PFH/DiD-GNPs particles were synthesized by a double emulsion technique. The average PLGA particle diameter was 560 nm, with 50 nm diameter silica-coated gold nano-spheres in the shell. MCF7 human breast cancer cells were incubated with PLGA/PFH/DiD-GNPs for 24 hours. Fluorescent and PA images were recorded using a fluorescent/PA microscope using a 1000 MHz transducer and a 532 nm pulsed laser. For the particle vaporization and drug delivery test, MCF7 cells were incubated with the PLGA/PFH-GNPs-PAC or PLGA/PFH-GNPs particles for 6, 12 and 24 hours. The effects of particle vaporization and drug delivery inside the cells were examined by irradiating the cells with a laser fluence of 100 mJ/cm^2, and cell viability quantified using the MTT assay. The PA images of MCF7 cells containing PLGA/PFH/DiD-GNPs were spatially coincident with the fluorescent images, and confirmed particle uptake. After exposure to the PLGA/PFH-GNP-PAC for 6, 12 and 24 hours, the cell survival rate was 43%, 38%, and 36% respectively compared with the control group, confirming drug delivery and release inside the cells. Upon vaporization, cell viability decreased to 20%. The particles show potential as imaging agents and drug delivery vehicles.

Keywords: PLGA particles, laser-induced vaporization, theranostic particles, photoacoustic, ultrasound, fluorescence, contrast agent, gold nanoparticles

1. INTRODUCTION

With the increasing incidence of fatal diseases like cancer, there is an urgent need for more precise methods of diagnosis and greater efficacy of therapy. Theranostic agents have been recently developed to simultaneously image and deliver therapeutic agents for combined diagnosis and therapy [1]. These multi-functional agents can be used for disease diagnosis and imaging applications via contrast agents with modalities such as OCT, MRI and fluorescence imaging. Further, therapy can be carried out using light-activated hyperthermia and by modulating the drug release rates based on the application and the polymer chosen [2]. These multi-functional nanoparticles could be used for chemotherapy and near infrared-based photothermal ablation of cancer cells. The results of *in vitro* and *in vivo* studies indicate that the combination of chemotherapy and photothermal therapies mediated by these nanoparticles provides a more effective treatment against cancer compared to either of these treatments alone [3].

Polymeric particles such as microspheres, microcapsules, nanoparticles fabricated using poly (lactide-co-glycolic acid) (PLGA) polymers are widely used for the delivery of a variety of drug classes due to their good biodegradable and biocompatible properties, and mechanical strength [4] [5]. The particles can also be used as a carrier to simultaneously deliver various chemotherapeutic drugs, imaging agents and targeting moieties. PLGA particles have been approved by the Food and Drug Administration (FDA) for drug delivery. Drugs formulated in polymeric devices are released either by diffusion through the polymer barrier, or by erosion of the polymer material [6].

In this study, we developed multifunctional nanocarriers for photoacoustic (PA) and ultrasound (US) imaging, and drug delivery vehicles. These nanocarriers were made of liquid perfluorohexane (PFH) and gold nanoparticles (GNPs) in the

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core and stabilized by a PLGA polymer as shells. The GNPs served as optical absorbers for PA imaging and as "fuses" for laser induced vaporization. Therapeutic drugs Paclitaxel (PAC) and fluorescent dye lipophilic carbocyanines DiD were loaded in the shell of the nanoparticles, and the particles were incubated with MCF7 human breast cancer cells. The PA and fluorescent images of cells containing PLGA/PFH/DiD-GNPs nanoparticles were obtained using a PA microscope with a 532 nm pulsed laser and high frequency (1GHz) ultrasound transducer, where the internalization of the particles by the cells was confirmed. Cell survival due to the effects of laser induced vaporization and drug release inside the cells was quantified using the MTT assay.

2. METHODS

2.1 PLGA/PFH-GNPs particle synthesis

Gold nanospheres (50 nm in diameter) were synthesized [7], then coated with silica [8], and fluorinated and dissolved into PFH liquid [9]. PLGA particles containing PFH and silica coated GNPs with and without GNPs were prepared using a double emulsion solvent evaporation process [10][11]. Briefly, PLGA polymer and DiD were dissolved in dichloromethane. GNPs in PFH solution were mixed with PLGA solution and emulsified using a digital sonifier (BRANSO, USA) equipped with a microtip. Then the emulsion was homogenized with polyvinyl alcohol solution. The final emulsion was mixed with a 2% isopropanol solution and stirred for 2 hours to vaporize organic solvents and washed several times by centrifugation. The final product of PLGA particles loaded with PFH and GNPs were collected and stored at 4°C for future use. To load the therapeutic drug PAC into the PLGA particles, PAC was dissolved in the PLGA in dichloromethane solution, and then the double emulsion method was followed.

2.2 Photoacoustic microscope

All PA measurements were obtained using a PA microscope (SASAM, Kibero GmbH, Germany). An inverted optical microscope (IX81 Olympus, Japan) with a transducer positioned above the sample stage and a focused laser (Teem Photonics, France) collimated through the side port onto the sample was used to record acoustic and photoacoustic signals. A 532 nm laser was used which had a 330 ps pulse width, 4 kHz repetition rate, a 1 μm focusing spot size, and a maximum energy of 10-nJ per pulse. The transducer used for this study has a central frequency of 1000 MHz, 42% bandwidth, and 60° aperture. Signals were amplified by a 40 dB amplifier (Miteq, USA) and digitized at 8 GHz (DC252, Agilent, USA) [12].

2.3 Photoacoustic and fluorescent imaging

A half million MCF7 cells were seeded in a 60 mm diameter glass-bottom cell culture dish. DiD labeled PLGA/PFH-GNPs particles were added into the dish and incubated for 24 hours. After the cells were washed with PBS several times, fluorescent dye Hoechst (40 μL, 10 μg/mL) and fluorescent dye lipophilic carbocyanines DiO (50 μL, 50 μg/mL) were added to the dish for 30 minutes. The cells then were fixed with 4% formaldehyde for 15 minutes. Then the cell culture dish was placed in the PA microscope. PA C-scan images of MCF7 cells containing PLGA/PFH-GNPs particles were obtained with a laser fluence of 45 mJ/cm^2. The fluorescent images were co-registered with the PA images and optical images.

2.4 Laser induced vaporization and drug release on the cell viability

MCF7 cells were seeded in a glass-bottom 96-well plate (8000 cells per well) and left overnight in the incubator. PLGA/PFH-GNPs (375 μg/mL) without and with PAC (7.5ug PAC/well) were added to each well according to the treatment and incubated for 6, 12, and 24 hours. Then each well was rinsed with PBS for several times to remove the free PLGA particles. A 532 nm pulsed laser (Minilite, Continuum, Canada) with a 3 ns pulse width, 10 Hz repetition rate, 100 mJ/cm^2 fluence, and 2 mm in diameter laser spot size was used to vaporize the particles. For the laser treatment groups, cells in each well were irradiated with the laser for 5 seconds at each spot and 9 spots in each well in total. After all the treatments were completed, the cell viability was tested using MTT assay.

3. RESULTS AND DISCUSSION

3.1 PLGA/PFH-GNPs particle synthesis

In this study, we used 50 nm gold nanospheres as the optical absorbing material due to their high absorption coefficient around 532 nm, and ease of synthesis [11] . A 20 nm silica coating was chosen to maximize the PA signal strength and the stability of gold nanospheres under laser irradiation [13]. Figure 1A shows the TEM image of a single PLGA particle

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containing GNPs and PFH in the core of the PLGA particle. GNPs are shown as black dots within the gray PLGA shell. Figure 1B is a SEM image of PLGA particles which demonstrate their spherical morphology. Figure 1C is the particle size distribution. The mean particle size is 560 ± 150 nm.

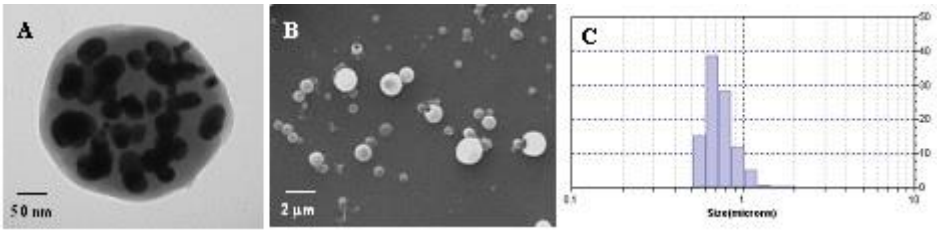

Figure 1. TEM image of a single PLGA/PFH-GNPs particle contains GNPs and PFH liquid (A). The black dots are the GNPs, and the gray pancake-shaped object is the PLGA shell. (B) This SEM image of PLGA/PFH-GNPs particles shows the spherical morphology of the particles. (C) The particle size distribution was 500 nm to 2 µm with a mean size of 560 ± 150 nm.

3.2 Photoacoustic and fluorescent imaging

MCF7 cells stained with lipophilic carbocyanines fluorescent dye DiO and Hoechst were incubated with DiD labeled PLGA/PFH-GNPs particles for 24 hours to investigate the PLGA/PFH-GNPs particle distribution inside the cells, and to investigate the potential of these particles as PA contrast agents. Figure 2 shows the representative optical, fluorescent, US and PA images of MCF7 cells loaded with PLGA/PFH-GNPs-DiD particles. In the superimposed fluorescence images, the cell nucleus stained with Hoechst is shown in blue, and the cytoplasm labeled with DiO is shown in green. The PLGA/PFH-GNPs labeled by DiD shown in red are located in the cell cytoplasm. In the superimposed PA images, cells generate strong PA signal (shown in red) at the location of the PLGA/PFH-GNPs. A PA signal around the other parts of the cytoplasm was not detected. The PA signal strength was generated by the GNPs located inside the PLGA particles.

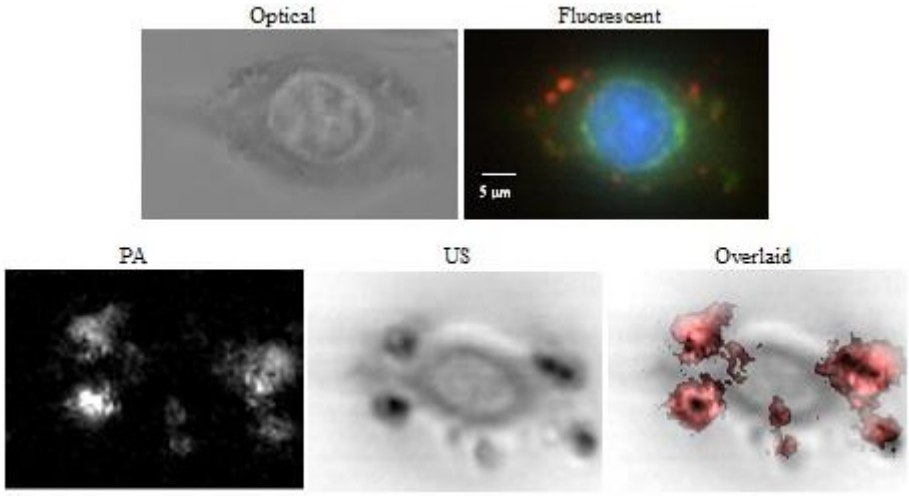

Figure 2. Images of MCF7 cells loaded with DiD labeled PLGA/PFH-GNPs. The optical image was recorded using a 20x phase contrast objective. In the fluorescence image, the cell nucleus is shown in blue due to the Hoechst stain and the cell cytoplasm is shown in green by DiO stain. The PLGA/PFH-GNPs particles are shown in bright white in the PA image, and in red in the fluorescence and superposed PA images.

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3.3 Laser induced vaporization and drug release on the cell viability

The MTT test results of laser induced vaporization and drug release inside the cells on the cell viability are shown in Figure 3. Laser irradiation alone does not change the cell survival rate. The exposure of PLGA/PFH-GNPs particles to the cells for 24 hours reduces the cell viability to 80%. After incubation with PLGA/PFH-GNPs particles loaded with PAC for 6, 12, and 24 hours, the cell survival rates are decreased to 43%, 38%, and 36% respectively compared with the control group. This result indicates that PAC was delivered inside the cell via the PLGA particle uptake by the cells and released through a diffusion or erosion process. The laser induced vaporization alone reduces the cell viability to 20% which demonstrates much stronger therapeutic efficiency than using PAC. The combination of using laser and PAC also reduces the cell viability to 20%. A synergistic effect of vaporization and drug release was not observed in this study. The reason might be due to the immediate cell death upon vaporization which overpowers the smaller cytotoxic effect of the low dose drug. In conclusion, this experiment shows that laser induced vaporization not only serves as a controlled drug release mechanism, but also serves as a powerful therapeutic tool for cancer treatment. The combination of utilizing laser and drug could achieve a desired therapeutic efficacy with lower doses of drug and reduction of side effect in patients due to the reduced systemic exposure to the drugs. The future work will be focused on the investigation of the synergistic effect of vaporization and drug release using different laser power levels and drug doses to achieve a maximal treatment effect.

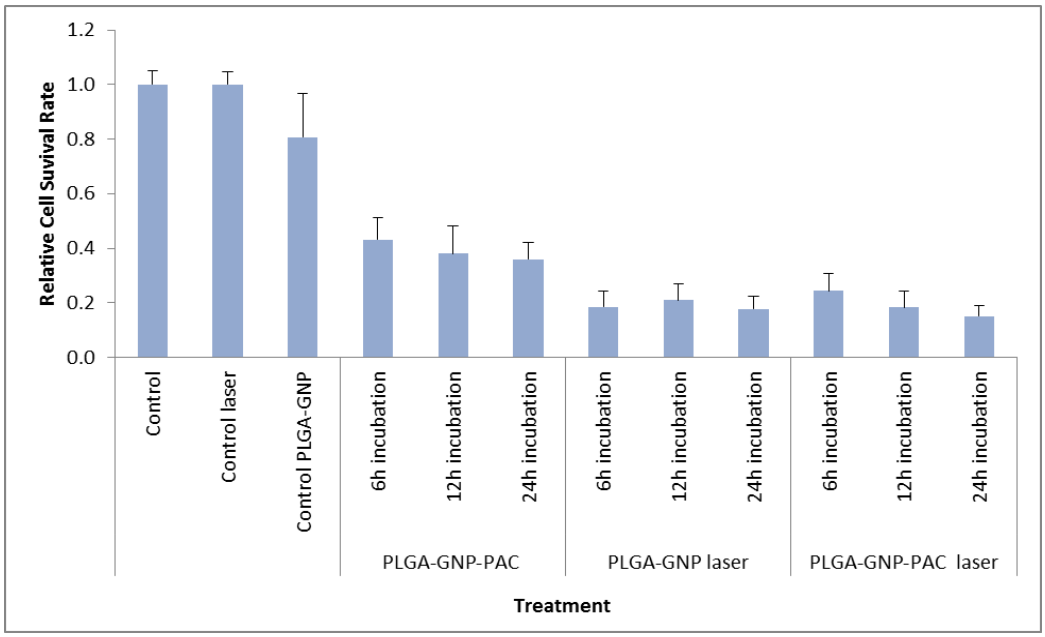

Figure 3. The effects of different treatments on cell viability were tested using MTT assay. The error bars are the standard deviations of 8 duplicates.

4. CONCLUSION

In this work, biodegradable PLGA particles containing silica-coated gold nanoparticles and fluorescent dye in the shell, and PFH in the core were developed as multifunctional agents for PA, US and fluorescent imaging. The therapeutic drug PAC was loaded into the particle shell and delivered into the cancer cells by endocytosis. The drug was either released through diffusion or vaporization. Vaporization via laser irradiation resulted in mechanical damage to the cells and reduced the cell viability dramatically. These agents show potential as multimodal theranostic agents allowing for US, combining fluorescent and PA imaging capabilities with therapeutic and drug delivery potential for cancer therapy.

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