Multifunctional perfluorocarbon nanoemulsions for cancer therapy and imaging

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ABSTRACT

There is currently interest in the development of nanoemulsions as imaging and therapeutic agents, particularly perfluorohexane (PFH) droplets, whose amphiphilic shell protects drugs against physico-chemical and enzymatic degradation. When delivered to their target sites, these perfluorocarbon (PFC) droplets can vaporize upon laser excitation, efficiently releasing their drug payload and/or imaging tracers. Due to the optical properties of gold, coupling PFC droplets with gold nanoparticles significantly reduces the energy required for vaporization. In this work, nanoemulsions with a PFC core and Zonyl FSP surfactant shell were produced using sonication. Droplets were characterized in terms of size and morphology using high resolution fluorescence microscopy (i.e. total internal reflection fluorescence microscopy, TIRFM), fluorescence correlation spectroscopy (FCS), transmission electron microscopy (TEM), and light scattering techniques (i.e. dynamic light scattering, DLS). The ability of PFC droplets to vaporize was demonstrated using optical light microscopy.

Keywords: PFH nanoemulsions, Droplet vaporization, Theranostics, Photoacoustic, Fluorescence spectroscopy/imaging

1. INTRODUCTION

Oral administration is one of the most widely used drug delivery methods because of its convenience and patient acceptance. However many important biological therapeutics are rarely used for oral drug delivery because of poor stability, solubility, and permeability. Better understanding of the characteristics and functions of these barriers have led to the design of nanocarriers which are able to increase the bioavailability of drugs. Recently, much attention has been focused on nanoemulsions (< 100 nm in size)[1,2,3]. Unlike other nanocarriers their liposome-like structure protects drugs against physico-chemical and enzymatic degradation. In addition, their charged surface promotes drug adsorption. One class of nanoemulsions called perfluorocarbon/perfluorohexane (PFC/PFH) emulsions are of particular interest because they combine drug carrying, tumor-targeting and imaging, enhancing both intracellular drug delivery and photoacoustic/ultrasound contrast imaging[4,5,6].

There are currently several techniques, including acoustic droplet vaporization (ADV) and optical droplet vaporization (ODV) for inducing a phase change in PFH nanoemulsions (PFH-NEs). In the first technique, a liquid droplet converts into a gas filled microbubble in response to a driving ultrasound pressure. This can lead to the phenomena called cavitation[7] where the microbubble expands and compresses over the duration of the transmitted pulse. This effect can provide better echogenicity for backscattering acoustic signals as well as improve drug release, for example to surrounding tissue in cancer tumor vasculature[4,7,8]. The second technique requires optical irradiation to cause droplet vaporization. Because of the high boiling point of PFH (56˚C), PFH droplets require optical absorbers [*i.e.*, fluorescent dyes, silica coated gold nanoparticles (scAuNPs), etc.] to transfer heat during vibrational relaxation to cause vaporization[9,10]. ODV of PFH nanoemulsions is preferred given that optical irradiation is less harmful to surrounding tissue, has relatively good penetration depth and allows for selective targeting of cell sub-populations, which can be used

Colloidal Nanoparticles for Biomedical Applications X, edited by Wolfgang J. Parak, Marek Osinski, Xing-Jie Liang,
Proc. of SPIE Vol. 9338, 93380R · © 2015 SPIE · CCC code: 1605-7422/15/$18 · doi: 10.1117/12.2079914

to exploit relevant time windows[11]. This work describes the synthesis and characterization of the perfluorocarbon droplets that are accompanied with the scAuNPs.

2. METHODS

2.1 PFH-NE/scAuNP preparation

The synthesis of gold nanoparticles (AuNPs) was carried out using the sodium citrate reduction method[12] by adding chloroauric acid ($HAuCl_4$) while boiling sodium citrate in Milli-Q water under vigorous stirring. The nanoparticles were confirmed to have negatively charged citrate ions by color change from initial yellow to a deep red. To coat the AuNPs with silica[13], (3-aminopropyl)trimethoxysilane (APS) was added along with a sodium silicate solution, whose pH was lowered to 10 for better polymerization onto the AuNPs.

PFH nanoemulsions were prepared by first vortexing the PFH, Zonyl FSP surfactant and water solution for 1 minute and then using a BRANSON microtip sonicator for emulsification (2 minutes sonication, pulsed at 10 seconds on/20 seconds off, 20% power amplitude, ~10 W) in an ice bath. The concentration of PFH and Zonyl FSP used to make PFH-NEs for all experiments were 12% (v/v) and 3% (v/v), respectively. Silica coated AuNPs (scAuNPs) were added after emulsification of PFH-NEs for fluorescence studies and to test for vaporization. For fluorescence imaging and spectroscopy, a TIRF microscope and a confocal system (both custom built) for FCS were used. The dyes DiO (3,3'-Dioctadecyloxacarbocyanine) and DiI (1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine) were added to the silica shell and PFH during synthesis to label the scAuNPs and PFH nanoemulsions. The particle size distribution, charge and morphology of nanoparticles were determined using dynamic light scattering (Brookhaven 90Plus), zeta potential (Brookhaven ZetaPlus) and transmission electron microscopy (JEOL JEM 1011 and 1200). Measurements were carried out on at least three independent replicate samples and standard deviation of their means reported. The optical absorption spectra of the scAuNPs was determined using a Perkin Elmer Lambda 20 UV/Vis spectrophotometer.

2.2 Photoacoustic/Fluorescence measurements

To determine whether PFH nanoemulsions could be vaporized, an inverted microscope (IX81 Olympus, Japan) was used. A 532 nm laser (Teem Photonics, France) was collimated through the side port and used for vaporization with 330 ps pulse width, 4 kHz repetition rate, 50 μm spot size, and an energy of 800 nJ per pulse (laser fluence of ~ 50 mJ/cm^2). A dichroic mirror (Chroma Technology Corp, USA) was used to reflect optical wavelengths between 450 and 620 nm, and transmit all other wavelengths for optical viewing. Measurements were made at physiological temperature (37°C) using the 40x phase contrast objective (Ph2, Olympus). For co-localization studies using TIRFM, the nanoparticles were immobilized onto a glass coverslip using a previously published PEG-Biotin-Streptavidin linker scheme[14,15,16]. For determining percent co-diffusion of particles using FCS, the raw intensity data was converted using a correlator and fitted to an autocorrelation model to determine the diffusion coefficient and amplitudes[17,18]. All fluorescence measurements were carried out using ~10 nM of sample to improve signal-to-noise and avoid detector saturation effects.

3. RESULTS AND DISCUSSION

3.1 Characterization of PFH-NEs/scAuNPs

Gold nanospheres were used because of their high optical absorption and ability to efficiently transfer heat to vaporize PFH nanoemulsions. It was found that without gold nanoparticles, PFH NEs could not be vaporized using the maximum laser fluence of the microscope. By adding a silica shell, gold nanoparticles are more stable under high laser irradiation[19]. The silica shell also enhances the photoacoustic signal, which will be important in future experiments testing the particles as photoacoustic contrast agents in cancer cell lines[20]. After synthesis, scAuNPs were found to have a maximum absorption wavelength close to the laser wavelength (Figure 1), ideal for vaporization experiments.

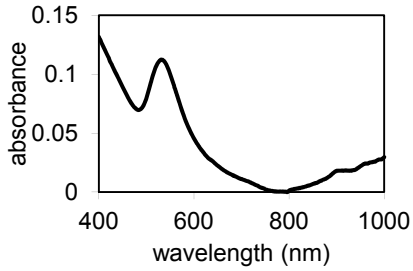

Figure 1. Characterization of scAuNPs. Typical absorption spectrum of silica coated gold nanoparticles (λ_{max} ~ 531 nm).

To characterize size and morphology of PFH-NEs, TEM and DLS were used. Electron microscopy shows that these nanoemulsions are spherical in shape with a PFH core (Figure 2A). The NEs were found to be very stable with an initial size of 52 ± 7 nm, increasing approximately by 10 nm by day 30 (64 ± 5 nm) (Figure 2B). This might be a result of its high negatively charged surface (-72 ± 5 mV zeta potential) (Figure 2C), preventing coalescence and flocculation.

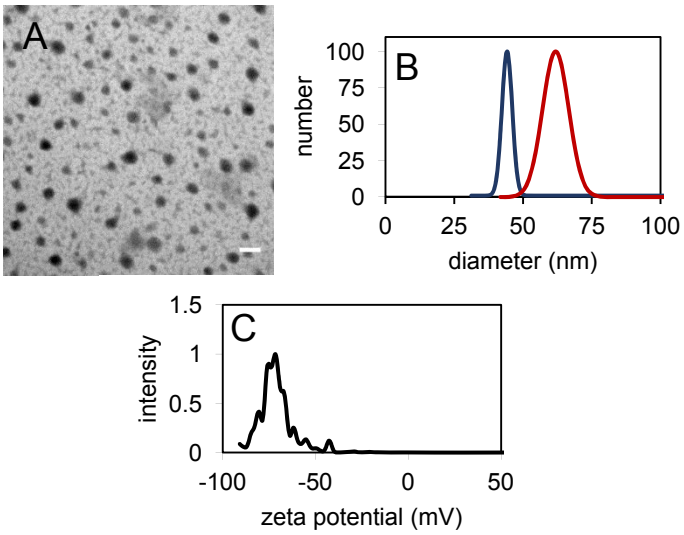

Figure 2. Size and stability of PFH-NEs. A) TEM image of PFH-NEs at day 1, scale bar: 100 nm; B) DLS measurement of particle size at day 1 (blue) and 30 (red); C) zeta potential of NEs (day 1).

3.2 Co-localization of PFH-NEs and scAuNPs

To label the core of PFH-NEs, the fluorescent dye DiI was first solubilized in diethyl ether prior to nanoemulsion formation so that DiI was miscible in PFH[21]. TIRFM showed a uniform size distribution of DiI labelled PFH-NEs with a significant amount of NEs bound to the coverslip using the PEG-Biotin-Streptavidin immobilization scheme. To determine co-localization of scAuNPs with PFH-NEs, DiI labelled PFH-NEs were first made and then mixed with DiO labelled scAuNPs. TIRFM images show that the PFH-NEs and scAuNPs are in very close proximity to each other, within the resolution limit of the microscope (~ 200 nm) (Figure 3). To determine the actual distance between PFH-NEs and scAuNPs, dual-color FCS (dcFCS) experiments were carried out and showed the presence of co-diffusing species from the fluorescent signal, as indicated by the blue crosscorrelation curve (Figure 4A). Incorporating the appropriate FCS crosscorrelation model, as discussed previously in literature[17,18], the percent of co-diffusing species was determined

to be 36 ± 5 % suggesting that scAuNPs are directly on the surface of PFH-NEs. TEM images of unlabelled NEs and NPs confirmed that scAuNPs are directly on the surface of PFH-NEs, likely due to electrostatic interactions (Figure 4B), with some NEs appearing bigger due to expansion.

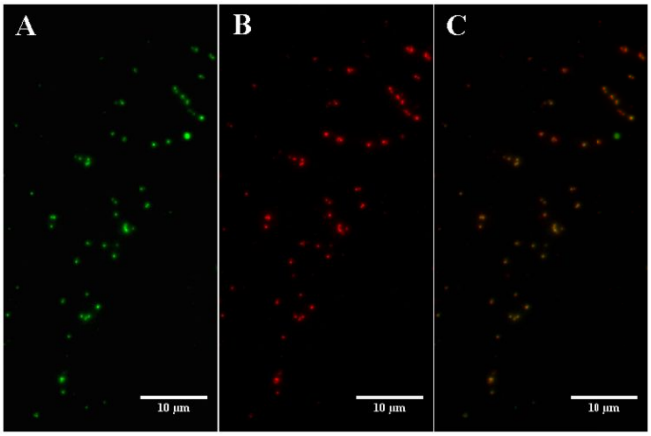

Figure 3. Dually labelled PFH-NEs and scAuNPs. TIRFM images of co-localization of PFH-NEs and scAuNPs. A) DiO labelled scAuNPs; B) DiI labelled PFH-NEs; C) co-localization of NEs and scAuNPs from overlapped images (A and B).

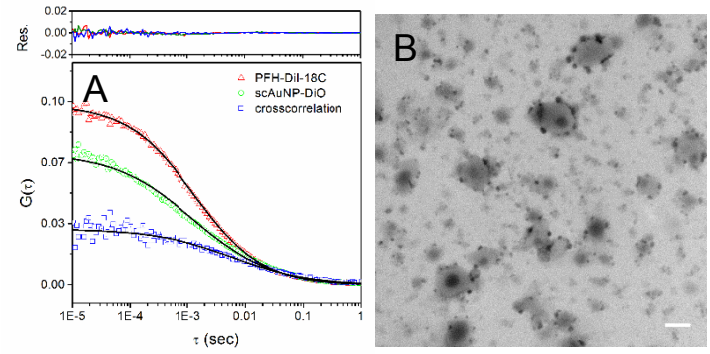

Figure 4. Co-localization and co-diffusion of PFH-NEs and scAuNPs. A) Dual-color FCS curve of DiO labelled scAuNPs and DiI labelled PFH-NEs; B) TEM image of non-fluorescently labelled PFH-NEs with scAuNPs, scale bar: 200 nm.

3.3 Vaporization of PFH-NEs

Optical microscopy showed that laser irradiation changed liquid PFH nanoemulsions into gas filled PFH microbubbles, in some instances greater than 10 μm in size (arrow, Figure 5B). The brighter spots seen in the image background might be from clusters of PFH-NEs and scAuNPs that are attracted to each other, with size distributions similar to those seen using DLS. The ability to vaporize PFH-NEs using a low laser fluence was likely a result of high concentration of scAuNPs used for experiments.

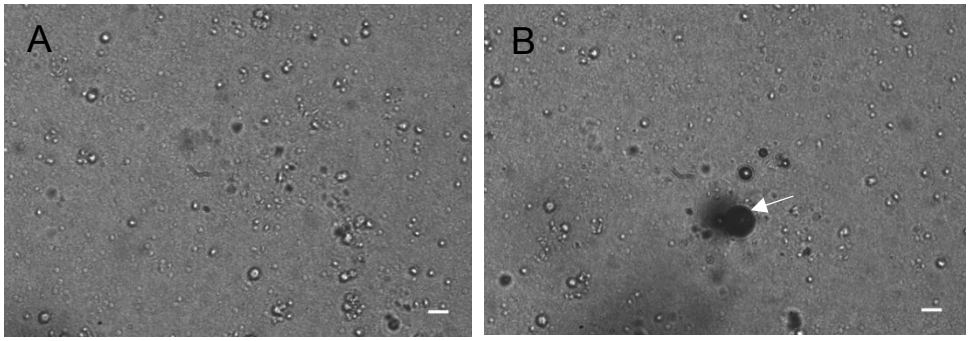

Figure 5. Vaporization of PFH-NEs. Optical images showing PFH-NEs with scAuNPs before (A) and 0.5 seconds after optical irradiation (B) using ~800 nJ/pulse and laser fluence of 50 mJ/cm^2, scale bar = 10 μm.

4. CONCLUSIONS

Monodisperse and very stable PFH nanoemulsions below 100 nm in size were created using ultrasonication. Silica coated gold nanoparticles were characterized in order to find the absorption maximum wavelength required to efficiently vaporize PFH-NEs. Fluorescence studies (TIRFM and FCS) and transmission electron microscopy showed that scAuNPs were on the surface of PFH-NEs with close to 40% of total particles co-diffusing. Vaporization of PFH-NEs showed that PFH microbubbles could expand to sizes greater than 10 μm before bursting. These PFH-NEs can potentially be used as theranostic agents to both improve contrast for photoacoustic and ultrasound imaging and efficiently release therapeutic drugs to target sites. Future experiments include testing drug loaded PFH-NEs in cancer cell lines to test therapeutic effects as well as see how vaporization affects cell integrity.

ACKNOWLEDGMENTS

This research was supported in part, by funding from the Canadian Institutes of Health Research (CIHR) awarded to M. Kolios. Equipment used to carry out experiments was purchased from grants from Canada Foundation for Innovation, the Ontario Ministry of Research and Innovation and Ryerson University.

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