Magnetoplasmonic Nanoplatforms for Enhanced Bioimaging and Photothermal Cancer Therapy

A. Kopwitthaya[1], T.Y. Ohulchanskyy[1], M. Jeon[4], W-C. Law[1], X. Xue[2], Q. Xie[3], R. R. Chada[3], E. P. Furlani[2,3], C. Kim[4], P. N. Prasad[1]

[1]Institute for Lasers, Photonics and Biophotonics, University at Buffalo, Buffalo, NY 14260, USA.

[2]Dept. of Chemical and Biological Engineering, [3]Dept. of Electrical Engineering, University at Buffalo, NY 14260, Office: (716) 645-1194 Fax: (716) 645-3822, efurlani@buffalo.edu

[4]Dept. of Biomedical Engineering, University at Buffalo, Buffalo, NY 14260, USA.

ABSTRACT

In this presentation we introduce a novel magnetoplasmonic nanoplatform for biomedical applications. The nanoplatform consists of a gold nanorod (GNR) and multiple Fe_3O_4 nanoparticles enclosed in a polymeric micelle-like structure. The combined magnetic and plasmonic functionality of the nanoplatform enables field-directed targeting, enhanced multimodal imaging, and therapy of diseased tissue. We demonstrate magnetophoretic control of the nanoformulations in vitro wherein an external field is used to accelerate uptake and aggregate the nanoplatforms in cancer cells. Following uptake, the nanoplatforms are illuminated with the femtosecond-pulsed IR laser, which causes photothermal destruction of the cells due to plasmonically-enhanced heating and bubble generation. We show that field-induced aggregation dramatically enhances the ability to selectively destroy targeted cells compared to the same therapy in the absence of a field. We also demonstrate that magnetically aggregated nanoplatforms produce enhanced photoacoustic tomography (PAT).

Keywords: Magnetoplasmonic nanoplatform, photothermal cancer therapy, plasmonic enhanced bubble nucleation, LSPR-induced absorption, photoacoustic tomography.

1 INTRODUCTION

Development of more effective cancer diagnosis and treatment modalities has challenged scientists for decades. Many attempts to this end have sought to exploit nanotechnology together with clinically-available imaging technologies such as computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) to facilitate cancer detection. Multifunctional nanoplatforms have showed promise in this field and are finding increasing use in biomedical applications for targeting, probing, imaging and treating tissue at the cellular and subcellular level. Many nanomaterials can function as both contrast agents for imaging and nanocarriers for cancer drug delivery. Examples of these include silica nanoparticles, quantum dots and magnetic nanoparticles. While many such particles are capable of increasing diagnostic sensitivity and prognosis, there are often limitations associated with random protein binding, biocompatibility and usage dose. Gold nanorods (GNRs) can overcome some of these limitations are finding increasing use due to their strong absorption in a NIR window, which provides an opportunity for deep tissue imaging. Recent reports have shown biocompatibility of surface modified GNRs in animals [1], GNRs are also prospective cancer therapeutic agents because of their photoinduced heat generation in the presence of pulsed-laser illumination, similar to other plasmonic nanostructures. The heat transfer from illuminated GNRs to the surrounding fluid can sufficient to generate supersonic nanobubble expansion, which can destroy cells [2]. The observed temperature increase is highest at the surface of the particle and falls off rapidly with distance [3]. This minimizes collateral damage to nearby normal cells, which is in contrast to conventional hyperthermia treatments.

Optical absorption is a key factor in the design of a suitable nanoplatform for photothermal therapy. While the heat generation is proportional to size of nanoparticles, large nanoparticles (>100nm) are obstructed by immune system [4]. However, effective heating can be achieved with smaller nanoplatforms by forcing them to aggregate. We propose to do this using magnetic field-induced clustering of magnetically functional nanoplatforms.

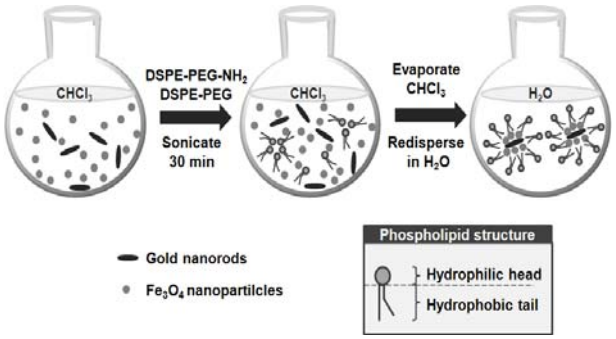
Previously, our group has proposed a multifunctional polymeric micelle-based nanocarrier for magnetophoretically guided drug delivery and photodynamic therapy [5]. The magnetophoretic control of the nanoplatform increases cellular uptake and provides enhanced imaging and phototoxicity in tumor cells. In addition, we have used a NIR dye encapsulated in polymeric micelle to provide high-contrast optical imaging and diagnosis of tumors in small animals [6]. These polymeric micelle-based formulations are stable and biocompatible. Recently, Qu et al. reported the noninvasive detection technique for nanoparticle endocytosis using magneto-photoacoustic (MPA) imaging [7]. They found an enhanced MPA signal as the concentration of magnetic

nanoparticles in macrophage cells increased. In addition, Mehrmohammadi et al. observed signal amplification from clusters of magnetic particles in comparison to the signal from individual nanoparticles using pulsed magneto-motive ultrasound imaging [8]. They further reported the use of pulsed magneto-motive ultrasound in the real time detection of intracellular accumulation of magnetic nanoparticles in macrophage cells [9].

In this presentation, the magnetic field-induced clustering of GNRs is demonstrated in vitro. The development of novel multifunctional magnetoplasmonic nanoplatforms for bioapplications will leverage the unique features of plasmonics for photothermal therapy and enhanced photoacoustic imaging, and nanomagnetics for field-directed targeting. The nanoplatforms are prepared using phospholipid (DSPE-PEG-NH_2 and DSPE-PEG) to encapsulate GNRs and iron oxide (Fe_3O_4) nanoparticles in a micelle-like structure. This nanoplatform has been characterized using transmission electron microscopy (TEM) to determine the morphology and size. Its absorption spectrum is used to determine magnetoplasmonic nanomicelle formation and nanoclustering. The nanoplatform exhibits an intense NIR (λ = 850 nm) absorption due to a longitudinal localized surface plasmon resonance (LSPR) of the GNR. This enables more efficient photonic stimulation and imaging in deep tissue due to the existence of the "transmission window" for biological tissues in the range of 650-900 nm. Dark field microscopy indicates enhanced cellular uptake of our nanoplatform in the presence of an applied field. Corresponding results have been observed via nanobubble nucleation in HeLa cells. Also, photoacoustic tomography has been demonstrated the high imaging-sensitive opportunity of this formulation.

2 RESULTS AND DISCUSSION

Phospholipid micelle-based magnetoplasmonic nanoplatforms were prepared using a mixture of GNRs and iron oxide (Fe_3O_4) nanoparticles in an organic solvent (Scheme 1). Facile and straightforward phase transfer of water-soluble GNRs into an organic phase solvent, i.e. chloroform, was carried out with the assistance of thiol-polyethylene glycol as GNRs surface ligand.



Scheme 1. Phospholipid micelle-based magnetoplasmonic nanoplatform (PGRFe) preparation.

Later, hydrophobic Fe_3O_4 nanoparticles were attached to GNRs surface following by phospholipid micelle (DSPE-PEG and DSPE-PEG-NH_2) wrapping in order to sustain water-soluble property in further biological use. The phospholipid based magnetoplasmonic nanoplatform (referred to as PGRFe) morphology and size were characterized using transmission electron microscope (TEM). The average dimension of GNRs is 57.05 ± 4.13 nm in long axis and 10.50 ± 0.45 nm in short axis (Fig.1A) while Fe_3O_4 nanoparticles have a mean diameter at 4.98 ± 1.44 nm (Fig.1B). After micelle formation, the anisotropic PGRFe size increases to 80 and 40 nm in long and short axis, respectively (Fig.1C and 1D). Fig.1E illustrates the extinction spectra which were records using UV-VIS spectroscopy. Red-shifted spectrum (~50 nm) was observed in PGRFe with maximum absorption peak at 850 nm indicated the micellar formation.

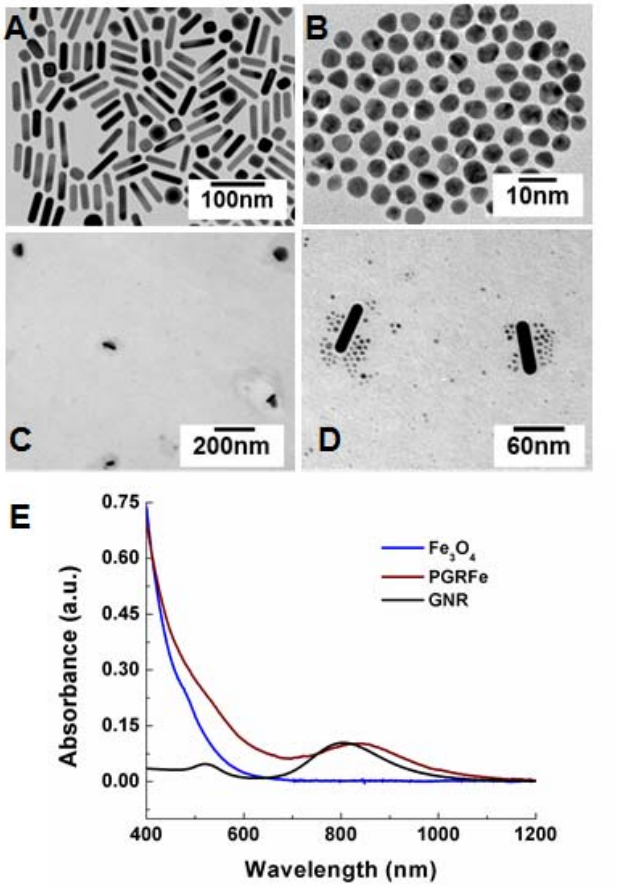


Figure 1. TEM images of (A) CTAB-GNRs, (B) Fe_3O_4 nanoparticles, (C) PGRFe with micelle contrast staining, (D) high magnification of PGRFe (E) extinction spectra of prepared nanostructures

The magnetic response experiment of PGRFe was performed using a cylindrical NdFeB magnet. After applying the magnetic field for 30 min, we observed a ring-like accumulation pattern, which indicates an increase of the PGRFe local concentration (Fig.2A). The local

concentration change of PGRFe under influence of magnetic field was also investigated using UV-VIS spectrophotometer (Fig.2B). By dynamic light scattering technique, the hydrodynamic diameter change under magnetic field demonstrated the nanocluster formation due to an applied field (Fig.2C) Hysteresis pattern of PGRFe was also studied to assure the retention of superparamagnetic property of PGRFe (Fig.2D). Beside the characterization and optical property study of prepared nanoplatform, the magnetic field distribution of magnet used in this experiment was calculated as well as strength of force exert on PGRFe by using numerical model. The magnet has a radius R_m= 12.5 mm, length L_m = 40 mm and magnetization M_s = 9.55×10^5 A/m. The magnetic field distribution in 2 and 3 dimensional spaces are shown in Fig.2E and 2F, respectively. Also, a profile of force is shown in Fig.2G and 2H.

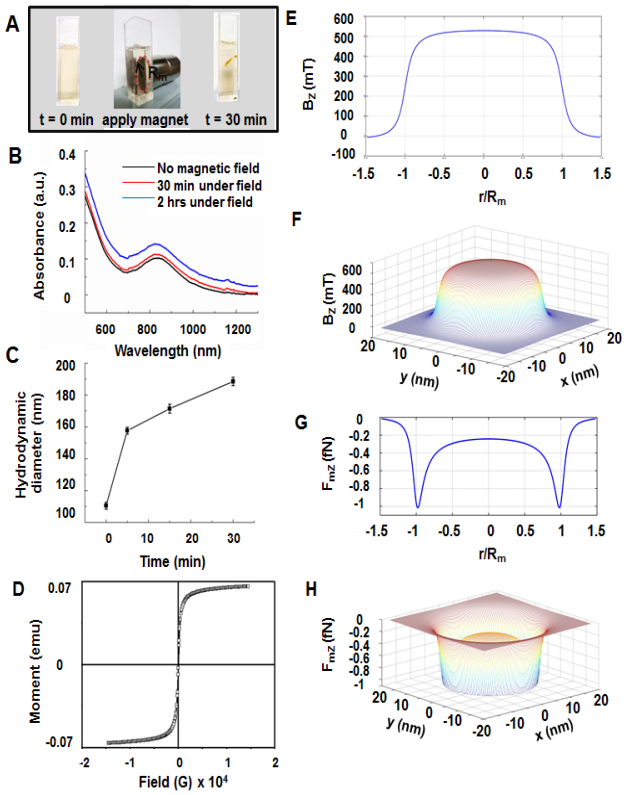


Figure 2. (A) Response of PGRFe to an applied field, (B) absorbance, (C) hydrodynamic diameter in an applied field, (D) hysteresis loop of PGRFe, (E) 2D and (F) 3D calculated magnetic field distribution of the laboratory magnet, (G) 2D and (H) 3D calculated magnetic force on PGRFe.

The increase in optical absorption due to an increase in the local concentration of the nanoplatforms in the presence of a magnetic field provides an enhanced photoacoustic (PA) signal at both 800 nm and 1060 nm excitation wavelengths (Fig.3). In the absence of magnetic field, PA signal of GNRs and PGRFe are comparable. Then, the applied magnetic field induces the PA signal enhancement up to 3 fold within 120 minutes compared with GNRs alone. Finally, the PA signal slowly decreases after magnet removal.

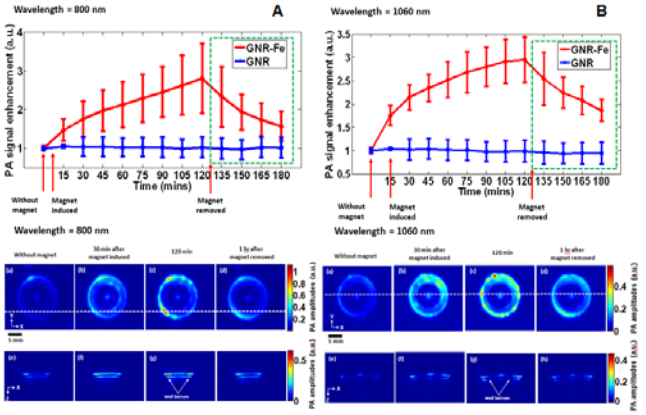


Figure 3. Photoacoustic signal enhancement of PGRFe at (A) 800 nm and (B) 1060 nm excitation.

The cellular uptake of the nanoplatform was investigated using ovarian human cancer cells, HeLa, as shown in Fig. 4. Field-directed PGRFe were two times more effectively internalized than folic acid targeted GNRs. Folic acid is known to increase cellular uptake due to its specificity in binding to folate receptor in ovarian cancer.

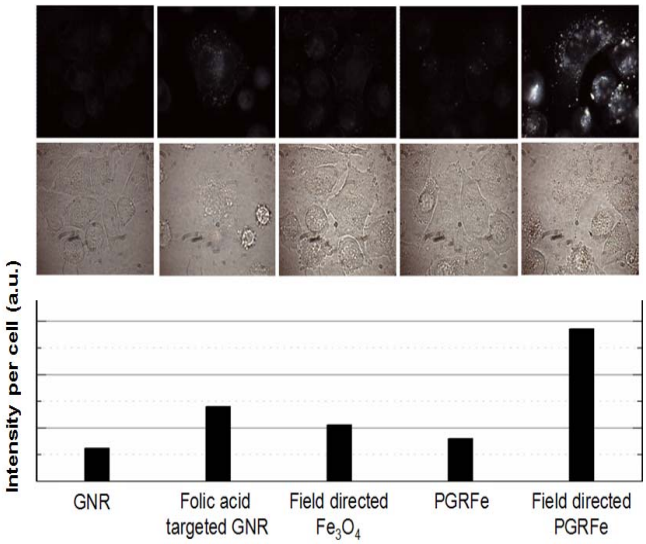


Figure 4. Cellular uptake efficiency in ovarian cancer cells.

Photoinduced thermalization has performed using a femtosecond-pulse laser at a wavelength of 780 nm. Figure 5 shows the nanobubble nucleation after irradiation with different laser powers. At a power below 3.2 $\mu W/\mu m^2$, there is a minimal photodestruction of the cells, even at 60 seconds of irradiation time. Bubble generation is clearly seen at power levels above 3.2 $\mu W/\mu m^2$. We observed the bubble generation in 10 seconds after expose to a laser at power of 6.4 $\mu W/\mu m^2$. Cell destruction was observed following bubble generation.

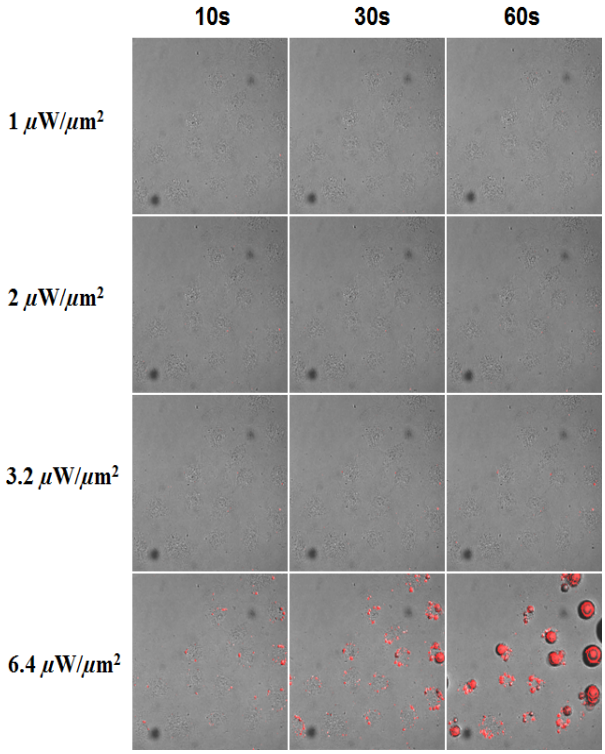


Figure 5. Power dependent plasmonic bubble generation in HeLa cells treated with PGRFe, incubation time 30 minutes (red indicates scattered light)

3 CONCLUSIONS

We have developed a novel nanoplatform for enhanced, magnetic field-directed, optically-based imaging and photoinduced therapy of cancer. This platform consists of a combination of GNRs and iron oxide nanoparticles within phospholipid micelles. Magnetophoretic control of the nanoformulation has been demonstrated, resulting in almost three-fold increase in a PAT signal in a magnetic field. Field-directed accumulation of the GNRs in cancer cells in vitro has been demonstrated, resulting in a substantial increase in optical imaging contrast and PPTT efficiency.

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