Near-infrared Fluorophore-doped Nanoparticles for *in vitro* and *in vivo* Bioimaging

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Abstract— Near-infrared (NIR) imaging technology has been widely used for bioimaging, since it can achieve deep penetration in biological tissues due to less absorption and scattering of NIR light. In our study, we reported the application of a kind of NIR fluorophore-doped nanoparticles for *in vitro* and *in vivo* microscopic bioimaging. Cellular imaging without autofluorescence, as well as vascular imaging of the mouse ear was obtained. The results of our experiments demonstrated NIR fluorophore-doped nanoparticles can be utilized as optical probes for high-contrast and deep-tissue functional bioimaging.

1. INTRODUCTION

Recently, optical imaging techniques have drawn great attention, due to their potentials for non-invasive study of living animals. Compared with visible light, near-infrared (NIR) light locating in "optical tissue window" (700–900 nm) [1] has less loss of light intensity and deeper tissue penetration capability. Thus, NIR imaging is a very powerful tool for deep-tissue in vivo bioapplications [2–4].

NIR fluorescent probes with high fluorescence efficiency, high chemical stability and less cytotoxicity are required in NIR imaging [5–9]. In our research, a typical type of NIR fluorophore, which is called IR-820, was adopted for NIR bioimaging. IR-820 doped organically modified silica (ORMOSIL) nanoparticles, as well as IR-820 doped polymer nanoparticles were synthesized, respectively. The nanoparticles have many advantages, such as relatively high fluorescence quantum yield, high stability in biological environment, facile surface functionalization and bio-compatibility, and they were further used for high-contrast *in vitro* cell imaging and deep-tissue *in vivo* animal imaging, separately.

2. EXPERIMENTAL SECTION

IR-820 doped ORMOSIL nanoparticles were synthesized according to the method previously reported [10]. 300 mg of Aerosol OT and 400 µl of 1-butanol were dissolved in 10 mL of DI water by vigorous magnetic stirring until the Aerosol OT was totally dissolved. 400 µl of the IR-820 in DMSO (0.5 mM) was then added to the solution. 20 minutes later, 100 µl of neat VETS was added into the mixture. After one hour, 15 µl of APTES was added to the solution. All the processes were under magnetic stirring for about 20 h. Aerosol-OT, 1-butanol, residual VTES and APTES were removed by dialyzing for at least 50 h. The dialyzed solution could be used for *in vitro* cell imaging after being filtered.

IR-820 doped polymer nanoparticles were prepared with the following method. 800 µl of DSPE-mPEG$_{5000}$ in chloroform (10 mg/mL) was mixed with 200 µl of IR-820 in ethanol (1 mM). The mixture was sonicated for several minutes to form a homogeneous solution and dried under vacuum in a rotary evaporator at 70°C. 250 µl of phosphate buffered saline (PBS, 10x) was added into the flask and the solution was sonicated for 2 minutes. Subsequently, an optically clear suspension containing IR-820 doped polymer nanoparticles was prepared, which can be used for further *in vivo* bioimaging.

HeLa cells were used for *in vitro* imaging, and nude mice were utilized for *in vivo* blood vessel imaging.

3. RESULTS AND DISCUSSION

A typical TEM picture (Figure 1) shows that IR-820 doped ORMOSIL nanoparticles were spherical, and have an average size of about 40 nm.

The absorption and fluorescence spectra of IR-820 doped ORMOSIL nanoparticles were shown in Figure 2. Their peak wavelengths are 835 nm and 850 nm respectively, which are located in a

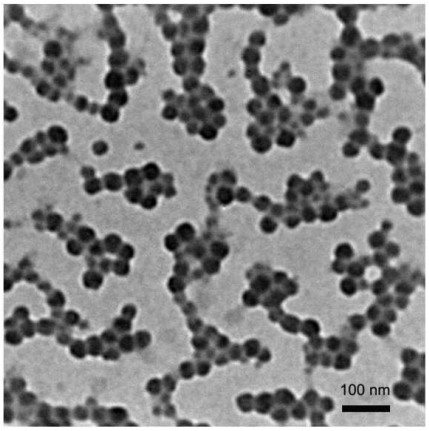

Figure 1: A TEM image of IR-820 doped ORMOSIL nanoparticles.

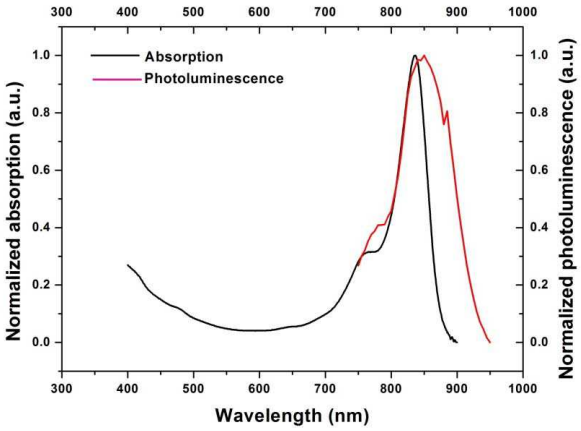

Figure 2: Normalized absorption and fluorescence spectra of IR-820 doped ORMOSIL nanoparticles.

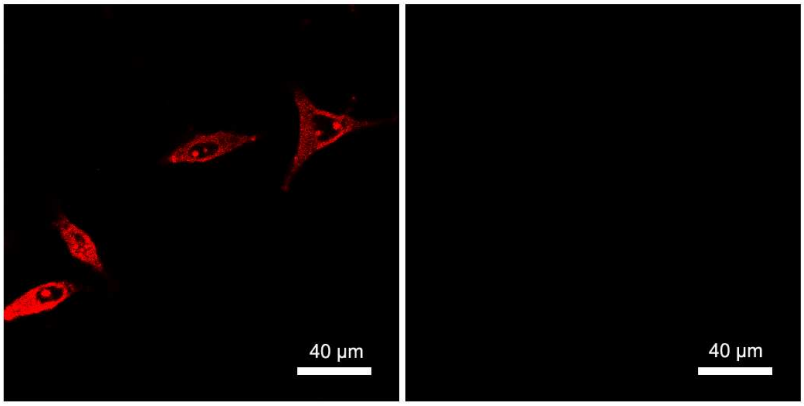

Figure 3: Fluorescence images of HeLa cells treated (a) with IR-820 doped ORMOSIL nanoparticles and (b) without IR-820 doped ORMOSIL nanoparticles (excitation wavelength: 635 nm).

NIR optical tissue window (700–900 nm), indicating that they can be applied in deep bioimaging with less absorption and scattering attenuation.

HeLa cells were cultivated for *in vitro* bioimaging and a 635 nm laser in an upright laser scanning confocal microscope (Olympus FV1000) was adopted as the excitation light source. Figure 3(a) is a high-contrast fluorescent image of HeLa cells, which were treated with IR-820 doped ORMOSIL nanoparticles for two hours. Due to bright fluorescence of IR-820 dyes under the 635 nm-excitation, as well as uniform staining of nanoparticles on cells, the morphologies of HeLa cells could be easily discriminated. For cells without the treatment of IR-820 doped ORMOSIL nanoparticles, nothing could be observed (Figure 3(b)), since the autofluorescence of HeLa cells was negligible under 635 nm-excitation.

Moreover, IR-820 doped polymer nanoparticles were utilized as optical contrast agents for *in vivo* bioimaging, due to their excellent photostability in animal body. The nanoparticles were intravenously injected into a nude mouse via the tail vein. The aforementioned upright laser scanning confocal microscope was used to perform *in vivo* imaging (excitation wavelength: 635 nm). Figures 4(a)–(h) show the images of blood vessels in the ear of the mouse at various vertical depths. Due to bright fluorescence of IR-820 and low background of tissue, the signal to noise ratio of all the images were very high, and even at 150 μm depth, the structure of blood vessels could still be observed very clearly. Furthermore, Figure 4(i) shows a 3D stack image (taken with 1 μm depth increment) of the NIR fluorescent nanoparticles in the blood vessels was reconstructed. Since the NIR fluorescence of the IR-820 was very distinct, the intravenously injected IR-820 doped polymer nanoparticles could reveal the vascular architecture very vividly. The experimental results

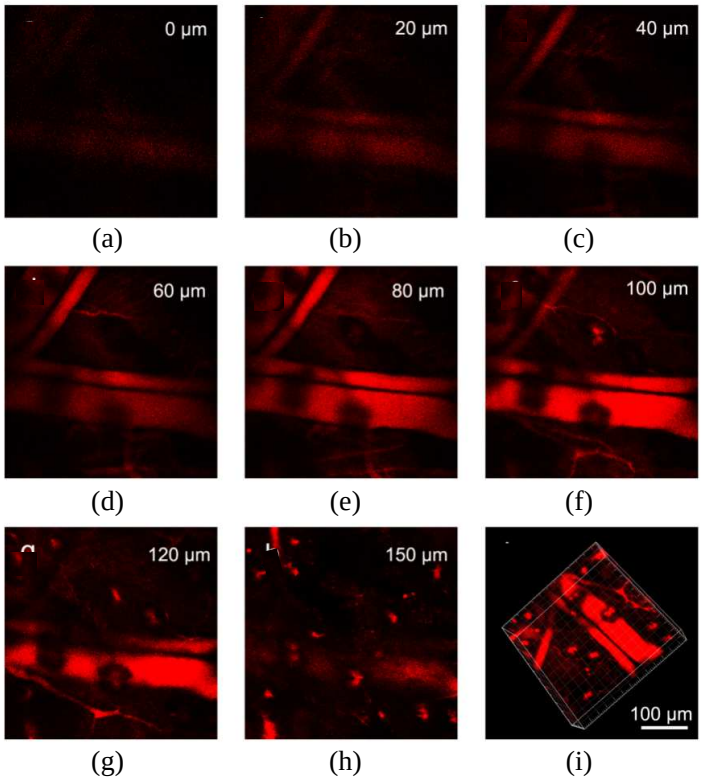

Figure 4: Images of blood vessels in the ear of a nude mouse at various vertical depths ((a) 0 μm, (b) 20 μm, (c) 40 μm, (d) 60 μm, (e) 80 μm, (f) 100 μm, (g) 120 μm, (h) 150 μm) and a three-dimensional reconstructive image (i) showing the distribution of the IR-820 doped polymer nanoparticles in the blood vessels of mouse ear.

illustrated that NIR fluorophore-doped nanoparticles have great potentials for high-contrast and deep-tissue *in vivo* functional bioimaging.

4. CONCLUSIONS

We demonstrated the applications of IR-820-doped nanoparticles as contrast agents for NIR optical imaging. High contrast living cells images, as well as deep-tissue (∼157 μm) vascular imaging in the ear of a mouse were obtained. Our study illustrated that NIR fluorophore-doped nanoparticles have great potentials for various *in vivo* functional bioimaging in the future.

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