Two-Photon Imaging and Photothermal Therapy of Cancer Cells Using Biofunctional Gold Nanorods

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Abstract: Transferrin-conjugated gold nanorods were used for targeting, two-photon imaging and photothermal therapy of cancer cells. The presence of nanorods significantly reduced the laser power effective for therapy.

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1. Introduction

Gold nanoparticles are good contrast agents for cancer detection attributable to their scattering and photoluminescence/fluorescence properties, as well as photothermal effects [1-2]. Among the gold nanoparticles of various shapes, gold nanorods attract special attention due to their longitudinal plasmon resonance at near infrared wavelengths, and the recent success in their size-controlled large scale synthesis [3].

Cancer therapy, especially targeted and localized therapy is a topic of current research interest. The advantage of the localized therapy is the minimal damage to the surrounding nonmalignant tissue. Attributable to the advance in detection of cancers at their very early stage, highly localized therapy becomes increasingly important. In addition, it is highly desirable that both imaging and therapy can be achieved with a single setup. In this work, two-photon imaging of the nanorods and therapy of HeLa cells were carried out using a pulsed femtosecond laser operating at 80 MHz. The wavelength was fixed at 800 nm. For imaging, an incident power of 700 µW was used to minimize the photothermal damage to the cells and nanorods. Using this system, both imaging and localized therapy was achieved by adjusting the laser power. In addition, two-photon excitation was used to image a nuclei acid stain, which makes the labeling of dead cells an easy task by changing the laser wavelength to 740 nm. Transferrin molecules were covalently conjugated to the surface of the gold nanorods to enhance their cellular uptake. Overexpression of transferrin receptors on HeLa cells has been reported [4]. Gold nanorods were prepared following a reported method [5]. To conjugate the nanorods with transferrin molecules, the surfactant molecules on the surface of the nanorods were replaced with thioglycolic acid molecules. A standard method was followed for the transferrin conjugation [6]. Polyethylene glycol molecules were also conjugated to the surface of the gold nanorods to stabilize them in culture buffer.

2. Results

Fig. 1a is a TEM image of the as-synthesized gold nanorods with an average length of 40 nm and an aspect ratio of 4. The absorption of the nanorods is centered at 800 nm (Fig. 1b).

![Fig. 1. A TEM image (a) and optical absorption (b) of the gold nanorods](image)
Fig. 2 shows a two-photon fluorescence image of the HeLa cell incubated with gold nanorods for six hours. The shapes of the cells can be clearly imaged with the aid of the transferrin-conjugated gold nanorods (Fig. 2a). In contrast, the cellular uptake of the unfunctionalized nanorods is much lower due to the nonspecific binding (Fig. 2b). The contrast indicates that transferrin-conjugated gold nanorods can be used for the targeting and imaging of this cancer cell line.

![Fig. 2 Two-photon images of HeLa cells having been incubated with gold nanorods for 6 hours. (a) Specific binding of transferrin-conjugated gold nanorods, (b) nonspecific binding of unconjugated gold nanorods. Scale bars: 20 µm.](image)

The photothermal effects of the gold nanorods on the HeLa cells are shown in Fig. 3. In the presence of gold nanorods, the cells can be killed after a few scans (10 seconds) at a very low laser power of 1 mW (Fig. 3b, nuclei of dead cells were stained with ethidium bromide). However, in the absence of gold nanorods, no cell death was observed after 1 minute’s laser scanning at a higher power of 3 mW (Fig. 3c and 3d). The results indicate that the presence of gold nanorods can significantly reduce the laser power effective for cancer therapy due to the photothermal effects of the nanorods.

![Fig. 3 Effects of nanorods on the photothermal therapy of HeLa cells. Ethidium bromide, a nucleic acid stain, was used to label dead cells. (a) with nanorods, before therapy, (b) with nanorods, after 10 scans at 1.0 mW, scale bar: 20 µm, (c) without nanorods, before therapy and (d) without nanorods, after 60 scans at 3 mW. All the pictures are on a same scale.](image)

3. References