Efficient NIR Hyperthermia and Intense Nonlinear Optical Imaging Contrast on the Gold Nanorod-in-Shell Nanostructures

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Gold nanorods (Au NRs) simultaneously act as NIR hyperthermia and nonlinear optical imaging agents, an important property for nanobiotechnology. Au NRs with strong surface plasmon resonance (SPR) in the NIR region have shown their potential in photo-induced therapeutic applications. The SPR of Au NRs can locally augment the field of incident electromagnetic waves, increasing the yield of nonlinear optical processes. Thus, Au NRs are ideally suited for nonlinear optical contrast agents in cell imaging using two-photon-excited luminescence. The two-photon fluorescence (2PF) from a single Au NR is 58 times that of a single rhodamine 6G molecule\textsuperscript{1} and provides cancer cell images to a depth of 75 µm.\textsuperscript{2} In order to develop metal nanomaterials with a strong SPR response when photo-excited and to preserve NIR absorption, biocompatibility, and easy surface modification, the new Au NR-in-shell nanostructures were developed to be more efficacious than Au NRs in NIR hyperthermia and nonlinear optical imaging contrast.

The Au NRs were served as seeds for the synthesis of Au NR-in-shell nanostructures(Figure 1a). Subsequently, an Ag nanolayer formed on the Au NRs because of the reactions of AgNO\textsubscript{3} and ascorbic acid, the resulting Au@Ag nanorods were shown in Figure 1b. The Au@Ag nanorods were allowed to react with HAuCl\textsubscript{4} aqueous solution, which yielded Au NR-in-shell nanostructures. Intact Au NRs were embedded in hollow Au/Ag shells with a shell thickness of ~4.8 nm (Figure 1c). High-resolution TEM indicates the (111) crystalline plane of the Au/Ag nanoshell (Figure 1c, inset). The energy dispersive X-ray analysis (EDX) results implied the shell should be Au/Ag alloy composition. The Au NR-in-shell nanostructures were well-dispersed in H\textsubscript{2}O. Figure 1d shows the extinction spectra of as-prepared Au NRs and Au NR-in-shell nanostructures measured with the same concentration of particles. The Au NR longitudinal SPR peaked at ~800 nm and the transverse SPR band centered at 520 nm; the Au NR-in-shell nanostructures had a broad band rising from ~400 nm to ~1100 nm, with the maximum at about 900 nm. The optical intensity of Au NR-in-shell nanostructures was much stronger than that of the Au NRs.
In the *in vitro* NIR hyperthermia experiments, the PEI/PSS modified Au NRs and Au NR-in-shell nanostructures were conjugated with anti-EGFR for specific targeting to the A549 cells overexpressing epidermal growth factor receptor (EGFR). The targeted cells were irradiated using an 808-nm CW laser. Cell damage caused by Au NRs was observable at 18 Wcm\(^{-2}\). On the other hand, Au NR-in-shell nanostructures destroyed malignant cells at half the laser power needed for Au NRs. Additionally, we found that the area of cells damaged by Au NR-in-shell nanostructures seems to be enlarged and larger than the laser beam spot. We used ICP to examine whether NIR laser irradiation caused Ag ions to leak inducing toxicity. We found that Au NR-in-shell nanostructures remained stable during NIR laser exposure. The reason for that Au NR-in-shell nanostructures caused damage beyond the laser beam size at this stage may be the incorporation of Ag in the Au NR-in-shell nanostructure.

Silver has greater thermal conductivity than gold does. The thermal conductivity for bulk Ag (429 Wm\(^{-1}\)K\(^{-1}\)) is larger than for bulk Au (318 Wm\(^{-1}\)K\(^{-1}\)).

The nonlinear optical properties of Au NRs and Au NR-in-shell nanostructures were examined using a home-built femtosecond (~100 fs) Cr:forsterite laser. The operating wavelength, 1230 nm, is in the biological penetration window with a deep penetration depth. The results show that Au NR-in-shell nanostructures...
generated multiphoton signals: second harmonic generation (SHG), third harmonic generation (THG), and multiphoton fluorescence signals (2PF, 3PF and 4PF). Such multi-resonant augmentation resulted in an ultra-broadband fluorescence from ~390 nm to near-infrared region. On average, the yield of the multiphoton signals of Au NR-in-shell nanostructures was average 55 times larger than that of Au NRs. The multiphoton-induced images of A549 cancer cells treated with anti-EGFR conjugated Au NRs and Au NR-in-shell nanostructures were taken in a multiphoton nonlinear microscope system (Figure 3). The 3PF (yellow), SHG band (green), 2PF (red) and THG (magenta) intensities of A549 cells treated with Au NR-in-shell nanostructures (Figure 3b) displayed more intense multiphoton signals with better contrast as compared to those of Au NRs (Figure 3a). The NRs distributed in the cells appeared as white spots in the merged image. The imaging signals for both materials were greater than those from cells alone. If A549 cells were treated with the amount of Au NRs 3 times Au NR-in-shell nanostructures, the multiphoton signals being comparable to those of Au NR-in-shell nanostructures were observed.

![Figure 3. Multiphoton images of (a) A549 cancer cells treated with Au NRs and (b) A549 cancer cells treated with Au NR-in-shell nanostructures. The yellow, green, red, and magenta colors indicate 3PF (450-600 nm), SHG band (605-625 nm), 2PF (>650 nm), and THG signals, respectively. The merged images from the four signals are shown in the bottom imaging frames of (a), and (b). The field of view is 16 X 16 μm.](image)

In summary, we synthesized Au NR-in-shell nanostructures and studied their capabilities as NIR hyperthermia and nonlinear optical imaging agents. The Au NR-in-shell nanostructures provided higher hyperthermia efficiency than Au NRs. The yield of multiphoton signals of Au NR-in-shell nanostructures was average 55 times larger than that of Au NRs, and the multiphoton images of Au NR-in-shell nanostructures have better contrast and stronger intensity than those of Au NRs.

References
