Gold Nanorod/Fe3O4 Nanoparticle "Nano-Pearl-Necklaces" for Simultaneous Targeting, Dual-Mode Imaging, and Photothermal Ablation of Cancer Cells

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Multifunctional Nanoparticles

Gold Nanorod/Fe₃O₄ Nanoparticle “Nano-Pearl-Necklaces” for Simultaneous Targeting, Dual-Mode Imaging, and Photothermal Ablation of Cancer Cells

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The combination of nanomaterials with different properties (such as magnetization, fluorescence, and near-infrared absorption) into one single object of nanoscale dimensions can lead to the development of multifunctional nanomedicinal platforms for simultaneous targeting, imaging, and therapy administration. Of the various kinds of nanoparticles available, magnetic nanoparticles (MNPs) have been utilized as versatile probes in biomedical applications, because of their utility in magnetic resonance imaging (MRI), quantum dots (QDs) and fluorescent-dye-doped silica nanoparticles are representative examples of nanoparticles used in optical imaging. In spite of their widespread use, fluorescent dyes are susceptible to photobleaching and quantum dots are difficult to functionalize in a controlled manner and are potentially toxic to cells, thus posing a concern for in vitro and in vivo applications. Recently, the photon luminescence effect of gold nanorods (Auₙ₉₉) has attracted much attention because of its excellent antiphotobleaching properties even under strong illumination and the chemically inert behavior of gold nanorods under physiological conditions, indicating that Auₙ₉₉ could be used as an imaging agent. Additionally, Auₙ₉₉ have been shown to exhibit excellent therapeutic properties as hyperthermal agents since the local temperature around the Auₙ₉₉ can be increased by laser illumination because of the tunable Auₙ₉₉ surface plasmon bands in the near-infrared (NIR) region. Among the various types of photothermal ablation technologies, NIR absorption photothermal therapy is particularly interesting because of the low scattering and low absorption by blood and soft tissue in this spectral region. The combination of MRI diagnosis (up to 25–100 μm spatial resolution), fluorescence imaging, and the NIR photothermal ablation (a few centimeters depth of that plume) could be used as an imaging agent to target cancer cells. An additional function of photothermal therapy will also be demonstrated.

Figure 1 illustrates the fabrication of novel “nano-pearl-necklace” structured multifunctional nanoparticles comprising a single, amine-modified Auₙ₉₉ decorated with multiple “pearls” of Fe₃O₄ nanoparticles capped with COOH groups. Then Auₙ₉₉–Fe₃O₄ nano-pearl-necklace (Auₙ₉₉–(Fe₃O₄)n) bioprobes for MRI, fluorescence imaging, and photothermal ablation of SK-BR-3 cells. "black bar: gold nanorod, CTAB: cetyltrimethylammonium bromide, gray spheres: Fe₃O₄ nanoparticles, EDC/NHS: 1-ethyl-3-(3-dimethylaminopropyl)carboimide hydrochloride/N-hydroxysuccinimide, Y: Herceptin."
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Figure 2. Transmission electron microscopic (TEM) images of A) COOH modified Fe₃O₄ nanoparticles, B) NH₂ modified Auₙ₋₄, C) Fe₃O₄ nanoparticles assembled onto the surface of Auₙ₋₄ stabilized by PEG (Auₙ₋₄-(Fe₃O₄)ₚ), D) UV/Vis-NIR absorption spectra (a–c) corresponding to samples (A–C). E) top: photograph of nanoparticles in water in the presence of an external magnet (a–c) corresponding to (A–C), and bottom: photograph of Auₙ₋₄-(Fe₃O₄)ₚ in different solvents, a) PBS, b) ethanol, c) chloroform, and DMSO, d) 1 M NaCl. F) Autocorrelation curve from fluorescence correlation spectroscopy (FCS) and photon lumination fluctuation trace (inset) measured from Auₙ₋₄-(Fe₃O₄)ₚ in water.

using EDC/NHS chemistry (see Figure 1). Figure 2C shows the corresponding TEM image of Auₙ₋₄ decorated with several Fe₃O₄ nanoparticles constituting the Auₙ₋₄-(Fe₃O₄)ₚ probe. Figure 2D a–c show the UV/Vis-NIR spectra of carboxylated magnetic nanoparticles, PEGylated amine-modified Auₙ₋₄ and PEGylated Auₙ₋₄-(Fe₃O₄)ₚ. The PEGylated amine-modified Auₙ₋₄ has a weak transverse plasmon (TP) band at 520 nm and a strong longitudinal plasmon (LP) band at 765 nm (Figure 2Db). After functionalization with Fe₃O₄ nanoparticles, the LP band is red-shifted from 765 nm to 785 nm and broadened (Figure 2Dc) reflecting a change in the local dielectric field resulting from the presence of Fe₃O₄ nanoparticles. Photographs in Figure 2E a–c illustrate the effective separation of PEGylated Auₙ₋₄-(Fe₃O₄)ₚ in the presence of an external magnet. PEGylated Auₙ₋₄-(Fe₃O₄)ₚ are much more easily separable by a magnet than free Fe₃O₄ nanoparticles because more magnetic nanoparticles are attached, the free Fe₃O₄ nanoparticles only respond weakly. Dynamic light scattering (DLS) measurement revealed that the hydrodynamic size of PEGylated Auₙ₋₄-(Fe₃O₄)ₚ in water was approximately 87 nm (Figure S1 in the Supporting Information), suggesting no apparent aggregation. The ability to vary the number of magnetic particles around a nanorod provides a magnetically tunable Auₙ₋₄-(Fe₃O₄)ₚ probe that can be used for ultra-sensitive MR diagnostic imaging.

Once fabricated, first the Auₙ₋₄-(Fe₃O₄)ₚ nanoparticles were separated from the unattached Auₙ₋₄ by an external magnet, then the free magnetic nanoparticles are removed by low-speed centrifugation. The Auₙ₋₄-(Fe₃O₄)ₚ nanoparticles were found to be stable in different solvents (phosphate buffer solution (PBS), pH 7.4, ethanol, chloroform, and DMSO) as well as in concentrated salt (1 M) solution (Figure 2E (bottom)). Interestingly, the Auₙ₋₄-(Fe₃O₄)ₚ also transfer partially into an organic phase in a chloroform–water system.

Fluorescence correlation spectroscopy (FCS), an ultrasensitive and non-invasive single-molecule technique generally used to study single-molecule diffusion and provide information on the number of molecules in a confocal volume (ca. femtoliter (fL)) by autocorrelating the fluorescence signal of the diffusers, was used to study the efficiency of these probes as a labeling and a targeting agent at single-molecule resolution. The diffusion characteristics of Auₙ₋₄-(Fe₃O₄)ₚ from the autocorrelation curve was fitted by a free 3D diffusion model[12] and the diffusion time was estimated to be 3.1 ms (Figure 2F). A typical count rate trace, depicting the fluctuation of Auₙ₋₄ fluorescence intensity in real time (Figure 2F, inset), suggests that these probes are stable and do not photobleach, ensuring their utility in imaging and single-molecule experiments.

The utility of Herceptin-tagged Auₙ₋₄-(Fe₃O₄)ₚ was demonstrated in dual-mode imaging and photothermal ablation of SK-BR-3 breast cancer cells overexpressing the human epidermal growth factor receptor 2 (HER-2). The remaining carboxy groups provided by Fe₃O₄ nanoparticles in Auₙ₋₄-(Fe₃O₄)ₚ were conjugated with Herceptin, which is a humanized IgG monoclonal antibody directed against the extracellular domain of the HER-2 receptor.[13] First the cytotoxic behavior of Auₙ₋₄-(Fe₃O₄)ₚ and Auₙ₋₄-(Fe₃O₄)ₚ-Herceptin conjugates on SK-BR-3 cells was examined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. As expected, the Auₙ₋₄-(Fe₃O₄)ₚ nanoparticles were reasonably nontoxic and biocompatible, whereas Auₙ₋₄-(Fe₃O₄)ₚ-Herceptin conjugates show a dose-dependent toxicity. Thus, no obvious cytotoxicity was observed at lower doses, while higher doses exhibited some toxicity was detected; Herceptin is known to have an anti-proliferative effect on HER-2 expressing cells[14] (Figure S2 in the Supporting Information).

To demonstrate the potential use as an enhanced MRI contrast agent, Auₙ₋₄-(Fe₃O₄)ₚ was compared to bare Fe₃O₄ nanoparticles of an equivalent iron concentration. MR images were obtained using a clinical 3.0-T MR system. The free Fe₃O₄ nanoparticles show a weak MR contrast with a r₂ relaxivity value of 63.5 mM⁻¹ s⁻¹; however, Fe₃O₄ nanoparticles decorating the gold nanorods, Auₙ₋₄-(Fe₃O₄)ₚ exhibited a remarkably stronger MR contrast with a dramatically increased r₂ value of 248.1 mM⁻¹ s⁻¹ (Figure 3A and Figure S3 in the Supporting Information). The significant improvement in the MR signal could be attributed to the synergistic magnetic effect of multiple Fe₃O₄ nanoparticles surrounding a core Auₙ₋₄ that result in magnetic coupling between Fe₃O₄ nanoparticles.[15] These results suggest that the Auₙ₋₄-(Fe₃O₄)ₚ has a stronger magnetization than free Fe₃O₄ nanoparticles and could serve as more effective contrast agents for MRI imaging of breast cancer cells. In Figure 3B (top), the T₂-weighted MR images for the untreated SK-BR-3 cells (a), the Auₙ₋₄-(Fe₃O₄)ₚ-Herceptin conjugates treated HER-2 MCF-7 cells (b), and Auₙ₋₄-(Fe₃O₄)ₚ treated HER-2 SK-BR-3 cells as a control are shown. The Auₙ₋₄-(Fe₃O₄)ₚ-Herceptin con-
jugates treated MCF-7 cells and control SK-BR-3 cells exhibited extremely weak MR signals as shown in Figure 3B (top; a, b, and c). The T2-weighted MR images of the Au rod–(Fe3O4)n–Herceptin conjugated probes targeting HER-2+ overexpressing SK-BR-3 cells show a much darker contrast as the probe concentration increased (Figure 3B, bottom).

The targeting specificity of Au rod–(Fe3O4)n–Herceptin nanoprobes for SK-BR-3 cells was assessed by the intracellular uptake of this nanoprobe compared to non-targeting nanoprobes as a control. A pronounced difference between the targeted and untargeted (that is, bare) nanoprobes against SK-BR-3 cells was noted. The change may be attributed to the ligand–receptor-mediated nanoprobe internalization by targeted cells.

By taking advantage of the inherent fluorescence of Au rod, fluorescent images from the Au rod–(Fe3O4)n probes were also obtained. The photon illumination confocal image of SK-BR-3 cells incubated with Aurod–(Fe3O4)n–Herceptin conjugates (Figure 4A) shows that some probes were bound to the cell surface and internalized into cytoplasm through specific binding to HER-2 overexpressing SK-BR-3 cells. Meanwhile, to further confirm that Aurod–(Fe3O4)n–Herceptin probes were specifically endocytosed by the SK-BR-3 cells, control experiments were performed using MCF-7 cells under these conditions. As expected, the nonspecific binding and the presence of endocytozed Aurod–(Fe3O4)n probes when MCF-7 cells were used was insignificant (Figure 4B). Control experiments using SK-BR-3 cells incubated with bare Aurod–(Fe3O4)n probes (i.e. without Herceptin) shows only the autofluorescence (Figure 4C), further confirming that these probes could be effecting labeling agents.

An autocorrelation plot of single nanoprobe (Figure 2F) diffusion using FCS (Figure 4D) shows that the intracellular diffusion time of Aurod–(Fe3O4)n was 5.5 ms (compared to the free diffusion time of 3 ms from Figure 2F)). The longer diffusion time may possibly be due to the high density of the cellular microcosm. These results suggest that the targeted Aurod–(Fe3O4)n–Herceptin could be very useful in cancer diagnostics and research.

The aforementioned results were also consistent with the cellular uptake shown in TEM images (Figure 5). Targeting of SK-BR-3 cells using PEGylated Aurod–(Fe3O4)n–Herceptin nanoprobes (1 h incubation at 37°C) shows that a significant amount of Aurod–(Fe3O4)n–Herceptin localized in the cytoplasmic vesicles (Figure 5A), while SK-BR-3 cells incubated with nanoprobes without Herceptin show no uptake for the same conditions (Figure 5B). These findings confirm that Herceptin-mediated endocytosis is a predominant mechanism for the uptake of PEGylated Aurod–(Fe3O4)n–Herceptin nanoprobes. More significantly, Figure 5A reveals that the Aurod–(Fe3O4)n–Herceptin nanoprobes maintain their original morphology and size within the cytoplasm of the cells assuring the integrity of these probes for multifunctional use. Effective internalization of the Aurod–(Fe3O4)n–Herceptin nanoprobes suggest that these probes can be used for targeting and as an inherent labeling agent. The integrity of these multifunctional probes further demonstrates that the controlled attachment of Fe3O4 nanoparticles around single Au rod could serve as an effective MRI contrast agent.
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To further confirm the feasibility of Au(rod)-(Fe3O4)–Herceptin nanoprobes as photothermal therapy agents, NIR irradiation-induced temperature changes for Au(rod)-(Fe3O4) nanoprobes (0.2 and 1.2 nm based on Au nanorod) were investigated. Results show an average temperature increase of approximately 20 and 25 °C for 0.2 and 1.2 nm Au(rod)–(Fe3O4), respectively, within 5 min. In contrast, pure water had no apparent increase after being exposed to laser irradiation (4.53 W cm⁻²) at the same condition (Figure S4 in the Supporting Information). These results show that Au(rod)-(Fe3O4) nanoprobes could be used as photothermal therapy agents.[16]

The HER-2 receptor is associated with homo and/or hetero dimerization of breast cancer cells by clustering on the plasma membrane. When Au(rod)-(Fe3O4)–Herceptin nano probes bind to HER-2 receptors at the cell surface, receptor clustering is expected to induce a large amount of nanoparticle internalization and consequently its aggregation in the vesicles and cytoplasm, as observed from TEM images (Figure 5A). Effective internalization provides an opportunity to utilize the same probes for the selective destruction of the vesicles and cytoplasma, as observed from TEM images (Figure 5A). Effective internalization provides an opportunity to utilize the same probes for the selective destruction of the vesicles and cytoplasma, as observed from TEM images (Figure 5A).

In conclusion, Herceptin-conjugated Au(rod)-(Fe3O4) nanoprobes were used to target SK-BR-3 cells. The multi-functional utility of the tumor targeting probes was successfully demonstrated by dual-mode imaging, single-nanoparticle intracellular dynamics monitoring, and photothermal ablation studies. The nanoprobes were shown to be magnetically and optically (in the NIR region) active and are therefore useful for simultaneous magnetic and optical detection. Further research on dual functionalization of these probes for targeted drug delivery and multimodal diagnostics in vivo is in progress.

Keywords: cell recognition • gold • imaging agents • nanoparticles • photothermal ablation


