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Gold Nanocages as Contrast Agents for Two-photon Luminescence Endomicroscopy Imaging

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Abstract

We explored the possibility of using gold nanocages as a new class of exogenous contrast agents for endomicroscopic nonlinear imaging. The two-photon luminescence cross-section of nanocages was characterized. Two-photon luminescence endomicroscopy imaging of phantom, cancer cells and tissues were performed, demonstrating Au nanocages can potentially serve as an effective molecular contrast agent for nonlinear endomicroscopy imaging.

Keywords

Endomicroscopy; Contrast agents; Gold nanocages; two-photon luminescence imaging

BACKGROUND

Two-photon fluorescence (TPF) endomicroscopy has excellent potential for translating the powerful TPF microscopy technology to clinical applications^{1,2}. The intrinsic TPF imaging contrast comes from two-photon-induced autofluorescence from endogenous fluorophores such as NADH, FAD and some structural proteins³. Most endogenous fluorophores, however, have a low two-photon absorption cross-section, i.e. on the order of 10^{-4} GM (GM= 10^{-50} cm⁴ s/photon), which limits their use for diagnostic imaging⁴. One remedy is to use exogenous dyes to provide a higher signal-to-noise ratio. Unfortunately most organic dyes also have a low two-photon absorption cross-section. Recently quantum dots have been explored for TPF imaging, which exhibit a two-photon cross-section much larger than organic fluorophores. The major challenge with most well-studied quantum dots is their toxicity, making them not suitable for *in vivo* clinical applications⁵.

Gold nanostructures can potentially overcome these issues, and have recently received increasing attention as a contrast agent for two-photon luminescence (TPL) imaging^{6,7}. Gold nanocages, as novel structured gold nanoparticles, have been reported as a contrast agent for OCT, photoacoustic and TPL microscopy imaging^{8–10}. Future *in vivo* applications of gold nanocages for TPL imaging, particularly for assessing internal organs, will require a miniature endoscope. The objective of this study was to investigate the feasibility of TPL

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imaging of gold nanocages using a recently developed fiber-optic scanning endomicroscope. Specifically, we demonstrated that two-photon endomicroscopy could directly examine the uptake of antibody-conjugated gold nanocages by cancer cells. We also performed TPL endomicroscopy imaging of biological tissues (such as liver and spleen) after intravenous administration of PEGylated gold nanocages, and the results show pronounced TPL contrast offered by gold nanocages.

METHODS

Synthesis of gold nanocages

The gold nanocages were synthesized using a well-established protocol¹¹, and the essential details are recapitulated in the Supplementary Materials section.

Fiber-optic nonlinear optical imaging endomicroscopy system

The TPL endomicroscopy system is illustrated in Figure 1. The detailed working principle of the scanning fiber-optic endomicroscope has been described elsewhere¹. In this study, we replaced the commonly used commercial double-clad fiber (DCF) with a customized DCF of a larger diameter inner-clad diameter (185 μm) to improve TPL signal collection. The microlens used at the end of the probe was a miniature, chromatic aberration corrected compound lens with a numerical aperture (NA) of 0.8, which offered a measured resolution about $0.76 \mu\text{m} \times 4.36 \mu\text{m}$ (lateral $\times$ axial), representing at least 2 times improvement over the previous endoscopes^{1,2}. The endomicroscope had a working distance of 200 μm (in water), resulting in a maximum imaging depth of $\sim 200 \mu\text{m}$. The overall probe head diameter is 2.0 mm (Figure 1A).

A tunable femtosecond Ti:Sapphire laser with built-in dispersion compensation from Coherent Inc. was used as the excitation source for TPL imaging. The incident power on the nanocage sample was moderate ($\sim 2 \text{ mW}$) with the TPL excitation wavelength (810 nm) close to the surface plasmon resonance (SPR) peak wavelength of the nanocages. The pulse width at the focus of the endomicroscope was about 250 fs. The schematic of the compact endomicroscopic TPL imaging system is illustrated in Figure 1B.

RESULTS AND DISCUSSIONS

Figures 2A and 2B show the SPR spectrum and TEM image of gold nanocages used in this study. The average edge length of the nanocages was $\sim 60 \text{ nm}$. Figure 2C shows a representative TPL endomicroscopy image of nanocages from a phantom. The nanocages produced a strong TPL signal, which was linear with the nanocage concentration (Supplementary Figure S1). As detailed in the Supplementary Materials section, the TPL cross-section of gold nanocages was found to be about $1.16 \times 10^7 \text{ GM}$ at 810 nm. This value is similar to the TPL cross-section of gold nanostars¹², but significantly higher than the TPL cross-sections of quantum dots ($5 \times 10^4 \text{ GM}$) and gold nanorods ($2.32 \times 10^3 \text{ GM}$)^{4, 6}. The results indicate that gold nanocages, in conjunction with all-fiber-optic scanning nonlinear optical endomicroscopy, could serve as a contrast agent for endomicroscopic TPL imaging.

To demonstrate the feasibility of gold nanocages as a contrast agent for endomicroscopy TPL imaging, A431 cancer cells were first incubated with nanocages which were conjugated with anti-EGFR antibodies. For bioconjugation, bi-functional molecules (NHS-PEG₂₀₀₀-OPSS) were used as a linker between the antibodies and gold nanocages, and the step-wise protocol is shown in Figure 3A. The surface of gold nanocages was further coated by PEG-SH to prevent aggregation. A representative TPL cell image is shown in Figure 3B, where cell membranes that overexpressed EGFRs and were targeted with antibody-conjugated gold

nanocages can be clearly identified. In comparison, no much TPL/TPF signals were observed from control cells that were not incubated with gold nanocages (Figure 3C).

To further test the feasibility of gold nanocages as a contrast agent for nonlinear endomicroscopy, we performed TPL imaging of biological tissues *ex vivo* such as liver and spleen, which were resected from a sacrificed mouse 12 hours after tail vein injection of 100 μ L of 0.2 nM solution of PEGylated gold nanocages. Representative images acquired with the endomicroscope placed on the liver or spleen surface are shown in Figure 4A. As a control, TPL imaging was also performed on the tissues after tail vein injection of saline. Strong TPL signals were detected from the liver and spleen of the mouse that received PEGylated gold nanocages (Figures 4A and 4B). In contrast, the two-photon auto-fluorescence signals from the control mouse liver and spleen (Figures 4C and 4D) were weak. These imaging results, along with the cell imaging results, demonstrate the feasibility that bioconjugated gold nanocages can serve as an effective molecular contrast agent for endomicroscopic TPL imaging.

In summary, bioconjugated gold nanocages were demonstrated as a molecular contrast agent for endomicroscopy TPL imaging. The results lay out a foundation for future translational TPL endomicroscopy imaging *in vivo* by using gold nanocages in conjunction with the fiber-optic endomicroscope.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

(A) Schematic of a piezoelectric (PZT) actuated fiber-optic scanning endomicroscope for TPL signal collection. The imaging speed was about 3 frames per second. (B) Schematic of endomicroscope system. PMT: Photomultiplier tube.

Figure 2.

(A) UV-vis-NIR spectrum of gold nanocages showing the SPR peak wavelength around ~790 nm. (B) Typical TEM image of gold nanocages with an average edge length ~60 nm. (C) Representative TPL image of gold nanocages in a phantom acquired by a scanning endomicroscope.

Figure 3. (A) Schematic of protocol using bifunctional molecules to conjugate antibodies onto gold nanocages, and the PEG-thiol to block the remaining surface. (B) TPL image of A431 cancer cells after incubation with bioconjugated gold nanocages. (C) TPF image of cancer cells as control which shows almost no detectable two-photon signal under 2 mW excitation.

Figure 4. TPL images of tissue sections. (A) Liver and (B) spleen after systemic administration of PEGylated gold nanocages. (C) Liver and (D) spleen after systemic administration of saline as control. The background TPF signals from the control (C and D) were only about 4% of the TPL signals when compared with (A and B).