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Theranostic Applications of Au-Ag Nanocages and Nanocomposites

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We report two novel biomedical applications of Au/Ag alloy nanoparticles called nanocages. In the first case, composite nanoparticles consisting of a nanocage core and a mesoporous silica shell doped with a photodynamic sensitizer Yb-2,4-hematoporphyrin were fabricated, characterized, and tested *in vitro* and *ex vivo*. In the second part of the study, Ag nanocubes and Au/Ag nanocages were applied to a multiplexed dot immunoassay. The assay principle is based on the staining of analyte drops on a nitrocellulose membrane strip by using multicolor nanoparticles conjugated with biospecific probing molecules.

Keywords: Nanocomposites, Dot-immunoassay, Nanocages.

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Multifunctional nanoparticles that combine therapeutic, diagnostic, and sensing modalities are a new trend in nanobiotechnology. One attractive option for theranostic applications are composites that combine the unique optical properties of plasmonic nanoparticles and the mesoporous silica functionalized with an appropriate photosensitizer (PS). In this work, we fabricated nanocomposites based on silica-coated gold-silver nanocages [1] functionalized with the photodynamic sensitizer Yb-2,4-dimethoxyhematoporphyrin (Yb-HP). The hybrid nanoparticles combine several promising theranostic modalities: (i) an easy tunable plasmon resonance across the 650-950-nm spectral band with possible use in photothermolysis; (ii) a mesoporous silica shell that preserves the plasmon resonance from an aggregation shift and provides a convenient possibility of surface or volume functionalization with various molecular probes; (iii) a combination of singlet oxygen generation with IR-luminescence band of Yb-HP, which can be used for optically controlled photodynamic therapy [2]. The nanocomposites had uniform distributions over the core and outer shell sizes, were stable in aqueous solution, demonstrated an absorption peak near 400 nm and two fluorescence peaks near 580 and 630 nm, corresponding to 4.8×104 bound Yb-HP molecules per one composite particle. Under 630 nm excitation, the composites generated singlet oxygen and produced heat under laser irradiation at the plasmon resonance wavelength (750-800 nm). The visible fluorescence, IR-luminescence, and singlet oxygen generation of bound Yb-HP molecules were not quenched owing to metal-PS interaction because of the separation from the nanocage core by the mesoporous silica shell. In particular, we observed enhanced killing of HeLa cells incubated with nanocomposites and irradiated by 630 nm light. An additional advantage of fabricated conjugates was an IR-luminescence band (900-1060 nm), originating from Yb3+ ions of bound Yb-HP and located in the long-wavelength part of the tissue transparency window. This modality was used to control the accumulation and biodistribution of composite particles in mice bearing Ehrlich carcinoma tumors in a comparative study with intravenously injected free Yb-HP molecules. Thus, these multifunctional nanocomposites seem an attractive theranostic platform for simultaneous IR-luminescence diagnostic, photothermal, and photodynamic therapy.

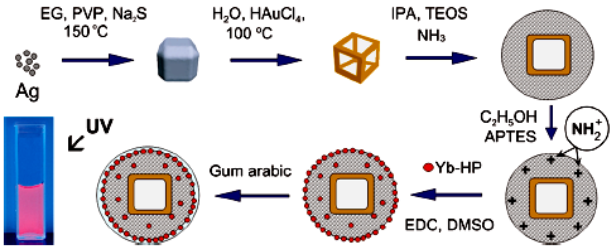

Fig. 1 – Schematic illustration summarizing how fluorescent composite nanoparticles can be fabricated starting with Ag nanocubes and ending with silica-coated Au-Ag nanocages functionalized with Yb-HP molecules. The left-bottom photo shows visible fluorescence of a sample under UV excitation. Designations: EG, ethylene glycol; PVP, poly(vinyl pyrrolidone); IPA, isopropyl alcohol; TEOS, tetraethyl orthosilicate; APTES, 3-aminopropyltriethoxysilane; Yb-HP, Yb-2,4-dimethoxyhematoporphyrin; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; DMSO, dimethyl sulfoxide

Alongside plasmonic nanoparticles, quantum dots (QDs) also serve as popular labels for multicolor optical imaging and immunoassay. However, the potential toxicity of QDs and the strong need for sample UV irradiation are major hurdles toward their widespread application, particularly *in vivo* or in nonequipped domestic circumstances (e.g., as a simple immunostrip assay for pregnancy diagnosis). On the other hand, metallic nanoparticles are better suited for such applications owing to their ease of synthesis, tunable plasmon resonance, and ready bioconjugation. Most importantly, they are less biotoxic [3] and do not require UV irradiation. Here, we report the first application of Ag nanocubes and multicolor Au/Ag alloy nanoparticles and nanocages to a multiplexed solid-phase dot immunoassay [4]. The assay principle is based on the biospecific staining of analyte

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drops on a nitrocellulose membrane strip by using a mixture of multicolor conjugates of nanoparticles functionalized with molecular probes. Although current synthesis protocols provide a great variety of nanoparticle sizes, shapes, and structures, the Au/Ag alloy nanoparticles seem a convenient platform for the multiplexed dot immunoassay. Indeed, the galvanic replacement reaction

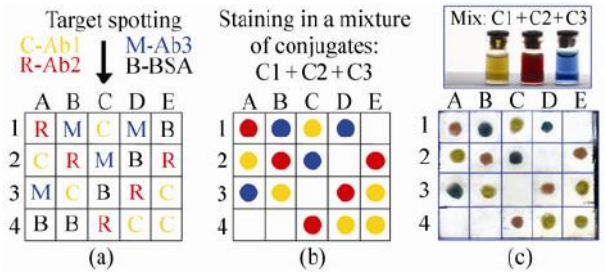

Fig. 2 – Figure 6 Schematic illustration of the multiplexed dot immunoassay (a, b) and its experimental verification (c). In the first step (panel a), anti-chicken rabbit antibodies Ab1 were spotted in squares A2, B3, C1, D4, and E3; anti-rat rabbit antibodies Ab2 were spotted in squares A1, B2, C4, D3, and E2; anti-mouse rabbit antibodies Ab3 were spotted in squares A3, B1, C2, and D1; and as a negative control, BSA was spotted in squares A4, B4, C3, D2, and E1. The concentration of all analytes was 100 μg/mL. After staining in a mixture of conjugates (C1 + C2 + C3), the expected spot colors are shown in panel b. The experimental panel c confirms the expected assay results

with silver nanocube templates [1] allows subtle and robust tuning of the Ag/Au conversion ratio and the nanoparticle suspension color from yellow to blue. With these nanoparticles in hand, we were able to conduct a multicolor dot immunoassay in a proof-of-concept experiment with three types of molecular targets and biospecific probes. Without any instrumental detection, the assay sensitivity was about 20 fmol under naked eye examination. Although this sensitivity is less than that of other techniques (e.g., ELISA, BIOCORE, or other biochemical methods), the simplicity and easy multiplex capability of our multicolor dot assay can find successful application in point-of-care and nonequipped circumstances (e.g., for quick and simple diagnosis of dangerous infections or multiple bacteria under field or domestic conditions). Furthermore, the sensitivity of our dot immunoassay can be improved by using silver enhancement and a flatbed scanner [5].

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