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EVENT ABSTRACT

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Fluorescent core-shell alloy nanoparticles for cell targeting applications

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Introduction: We report the synthesis and characterization of gold-silver (Au/Ag) alloy core silica shell (Au/Ag@SiO2) nanoparticles (NPs) incorporating fluorescent molecules and functionalized with antibodies (Abs) to target cancer cells (Fig. 1). These multifunctional Au/Ag@SiO2 NPs present various advantages: (1) The size of the Au/Ag core can produce tunable scattering signals due to local plasmonic resonance effect which can be easily collected by darkfield microscopy[1]; (2) The Abs-functionalized NPs can target cell surface receptors as contrast agent in flow cytometry (fluorescence and scattering channels)[2] due to high optical cross-section; (3) The field enhancement around the metallic core can increase the intensity of fluorescent molecules since they have a higher excitation and emission rate, generate more photons and are less vulnerable to photobleaching than unprotected fluorescent molecules[3]-[5]; (4) The combination of multiple NPs displaying characteristics signals in both darkfield and fluorescence channels can bring a significant improvement in terms of marker efficiency; (5) The SiO2 shell should allow the incorporation and protection of a high number of fluorescent molecules and further ease the surface modification.

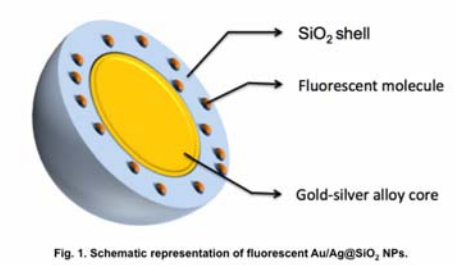

Fig. 1. Schematic representation of fluorescent Au/Ag@SiO_2 NPs.

Material and Methods: Fluorescent molecules (Fluorescein isothiocyanate (FITC), Rhodamine B isothiocyanate (RBITC) and CF 647) were incorporated in the SiO2 shell after reaction with (3-aminopropyl)triethoxysilane (APTES) diluted with dimethylformamide (DMF) and triethylamine (as a catalyst) to form fluorescent precursors (FPs). Au/Ag@SiO2 NPs were synthesized with tetraethyl orthosilicate (TEOS), ammonia solution, FPs and 60 nm citrate-capped (25/75 or 50/50) Au/Ag alloy NPs diluted in EtOH under continuous stirring for 24 h. Au/Ag@SiO2 surface was modified with (3-mercaptopropyl)trimethoxysilane (MPTMS) to react with Abs (anti-CD44 and anti-EGFR as positive Abs, anti-Nectin2 as negative Abs) in presence of sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC). Functionalized NPs were incubated with human HeLa cancer cells for 30 minutes in dark environment on ice.

Results and Discussion: Fluorescent tunable Au/Ag@SiO2 NPs have been synthesized and characterized (Fig. 2). NPs containing 60 nm Au/Ag (25/75 or 50/50)[6] have been produced and visualized by TEM (Fig. 2A,B) since their different scattering peaks can be used in darkfield microscopy. The fluorescent molecules incorporated in the NPs showed clear signal compared to bare Au/Ag NPs and supernatant (Fig. 2C-E). Au/Ag@SiO2 NPs containing FITC were then observed by darkfield and fluorescence microscopy (Fig. 2F,G). The scattering and fluorescent signals colocalized and the higher scattering intensity of NP clusters colocalized with brighter fluorescent signal. The fluorescent NPs targeting cancer cells was confirmed by flow cytometry (Fig. 3).

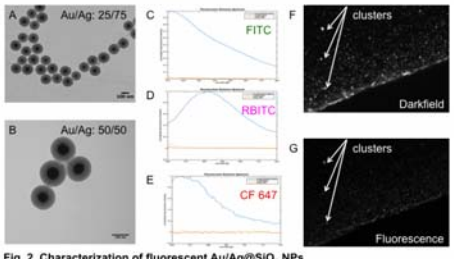

Fig. 2. Characterization of fluorescent Au/Ag@SiO_2 NPs.
Structure of 60 nm Au/Ag (25/75 (A) and 50/50 (B)) core with 40 nm SiO_2 shell observed by TEM. The fluorescence spectra of FITC (C), RBITC (D) and CF 647 (E) incorporated in Au/Ag(25/75)@SiO_2 NPs compared to the spectra of bare Au/Ag NPs and supernatant measured by UV-visible spectroscopy. Darkfield (F) and fluorescence (G) microscopy of Au/Ag@SiO_2 NPs containing FITC (60x magnification).

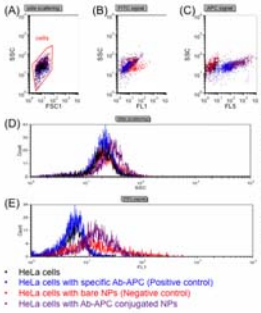

Fig. 3. Cell targeting with fluorescent Au/Ag@SiO_2 NPs. Flow cytometry data showing the increase of side scattering and FITC (FL1) signal of NPs tagged cells. Allophycocyanin (APC) is used as an indicator of Ab functionalization on NP surface. The broad distribution of positively labeled cells in APC (FL5) channel indicates the different number of NPs on each cell, which also correspond to the side scattering channel due to the high scattering signal of NPs. From FL1 channel we observe also the nonspecific targeting of bared NPs.

Conclusion: Fluorescent Au/Ag@SiO2 NPs targeting different cell surface receptors were synthesized, characterized and used as contrast agent labeling cancer cells. The combination of scattering and fluorescence channels in flow cytometry from the proposed NPs incorporating different fluorescent molecules would allow multifunctional and multichromatic possibilities for in vitro and in vivo imaging.

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