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SHORT COMMUNICATION

Metal Enhanced Fluorescence Solution-based Sensing Platform 2: Fluorescent Core-Shell Ag@SiO_2 Nanoballs

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Abstract In this Rapid Communication, we present the development of monodisperse core-shell (silver core-silica shell) nanoparticles with various shell thicknesses featuring a fluorophore, subsequently named Metal-Enhanced Fluorescence (MEF) nanoballs. MEF nanoballs consist of *a* ≈130 nm silver nanoparticle core, a silica shell with up to 35 nm thickness and fluorophores doped within the silica shell. Fluorescent nanobubbles where the silver core is removed by chemical etching are used as control samples to show the benefits of using silver nanoparticles, i.e, Metal-Enhanced Fluorescence. Finally, we demonstrate the broad potential biological applications of MEF nanoballs by employing near-infra red emitting probes (Rhodamine 800) within the silica shell, for potential applications in cellular imaging and solution-based sensing.

Keywords Metal-enhanced fluorescence · Radiative decay engineering · Plasmon enhanced luminescence · Plasmon enhanced fluorescence · Surface enhanced fluorescence · Fluorescence · Plasmon · Plasmonics · Nanoparticles · Silver nanoparticles · Silver colloids · Solution sensing platform

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Symbol and acronyms

Ag@SiO_2	Silver core, silica shell nanoparticles
MEF	Metal-Enhanced Fluorescence
SPR	Surface Plasmon Resonance
Rh800	Rhodamine 800
TEM	Transmission Electron Microscope
TEOS	Tetraethyl orthosilicate

Introduction

Metal-Enhanced Fluorescence (MEF) is a powerful technology [1], where the interactions of fluorophores with metallic nanoparticles results in fluorescence enhancement, increased photostability, decreased lifetimes due to increased rates of system radiative decay [2] and increased transfer distances for fluorescence resonance energy transfer [3]. However, almost all of the MEF applications to date have been performed on 2-dimensional surfaces, where glass microscope slides [4–6] or plastics [7, 8], are used as the primary substrates that feature the silver nanostructures deposited using either wet-chemistry [4–8], electrochemically [9] or lithographically [9].

In 2004, we made the first attempt to utilize silver nanoparticles in a solution-based enhanced fluorescence sensing platform, where we typically observed a 3–5 fold enhancement in fluorescence from the biotinylated silver core-silica shell nanoparticles and by their aggregation with Cy3-labeled streptavidin in suspension [10]. The thrust of that study was geared towards fluorescence-based aggregation assays, where the aggregating silver nanoparticles resulted in enhanced fluorescence emission due to enhanced electric fields between the nanoparticles. Given these promising results for solution-based MEF assays, we subsequently continued our efforts using the core-shell nanoparticle architecture

for solution-based MEF sensing. In this regard, we have developed a new class of fluorescent core-shell $Ag@SiO_2$ nanoparticles, or simply called MEF nanoballs.

MEF nanoballs are comprised of a silver core, and a silica shell with fluorophores embedded within the silica shell. Transmission Electron Microscope (TEM) analysis of the MEF nanoballs has shown that the silver core was ≈130 nm diameter, while the thickness of the shell could be varied from between 2 to 35 nm, in controlled procedures. After the construction of the silver core-silica shells, fluorophores were simply doped into the silica shell. In order to show the benefits of using a silver core, we have also prepared control sample fluorescent nanoparticles, called fluorescent *nanobubbles*, made from the MEF nanoballs: the silver core of the MEF nanoballs chemically etched with a solution of sodium cyanide which yielded a hallow fluorophore-doped silica shell, appropriately called fluorescent *nanobubbles*. Since the fluorophore used here in the MEF nanoballs, Rhodamine 800 (Rh800), is hydrophobic in nature [11], it is trapped within the hydrophobic pockets of the silica shell, where the chemical etching of the MEF nanoballs did not displace (or quench) Rh800 from the shell. Hence, the extent of fluorophore loading into the MEF nanoballs and in the fluorescent nanobubbles was identical, making the nanobubbles an excellent control sample and importantly, making it possible to compare the emission of the fluorescent MEF nanoballs with the fluorescent nanobubbles in a *quantitative* manner. Subsequently, we have observed a 20-fold larger fluorescence emission from the MEF nanoballs than that of the nanobubbles. This enhancement is due to the close proximity of the fluorophores to the silver core, i.e Metal-Enhanced Fluorescence [1–10].

Materials and methods

Materials

Tetraethyl orthosilicate (TEOS), silver nitrate, sodium citrate, iso-propanol, and ammonium hydroxide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Rhodamine 800 (Rh800) was purchased from Lambda Physik (Fort Lauderdale, FL).

Methods

Preparation of the Fluorescent Core-Shell $Ag@SiO_2$ Nanoballs (MEF Nanoballs)

Preparation of silver colloids: Silver colloids were prepared by adding dropwise 10 mL of 38.8 mM sodium citrate aqueous solution within 2 minutes to 490 mL of boiling aqueous solution containing 90 mg of $AgNO_3$ under vigorous stirring. After boiling for 10 minutes the solution was cooled to room temperature. The as-prepared silver colloid solution was centrifuged at 500 rpm for 1 hour to remove larger colloids, the remaining silver colloids in solution having an average size of ≈130 nm, as confirmed by TEM.

Preparation of core-shell $Ag@SiO_2$ nanoballs: Under vigorous stirring, 1 mL of silver colloid solution was mixed with 250 mL of iso-propanol and 25 mL of deionized water. Immediately after the addition of 4 mL of 30% ammonium hydroxide, different amounts of TEOS were added to the reaction mixture. To obtain different silica layer thicknesses, 100 μl of the same amount of TEOS solution with a concentration between 50% and 100%, was added to the suspension. The reaction was stirred at room temperature for 30 minutes and then was allowed to age without agitation at 4°C overnight. Each suspension of $Ag@SiO_2$ nanoballs was washed and centrifuged (at 3500 rpm for 30 min) three times with a water ethanol mixture (5:4) for 30 min, followed by resuspension in water. The thickness of the silica layers was determined from TEM images and varied from 2 to 35 nm, depending on the preparation parameters.

Preparation of the fluorescent MEF nanoballs: The fluorescent MEF nanoballs were prepared by dyeing (doping) of the $Ag@SiO_2$ nanoballs. 500 μl ethanol solution, 0.088 mg/ml Rh800, was added to 500 μl (0.2 mg) of $Ag@SiO_2$ nanoballs suspended solution and incubated overnight. The mixture was centrifuged and washed with 1.5 ml H_2O and 1.2 ml ethanol 4 times, respectively. The absorbance and the fluorescence spectra of the washing solution were monitored to ensure the complete removal of the *unadsorbed* fluorophores.

Preparation of the nanobubbles from the MEF nanoballs: The nanobubbles prepared from the fluorescent MEF nanoballs were obtained using the following procedure. 500 μl of 0.1 M sodium cyanide solution was added to an aged suspension of 200 μl of MEF nanoballs with agitation overnight to dissolve the silver core of the particles. Each suspension of MEF nanoballs/nanobubbles was washed and centrifuged three times with 1.5 ml water and 1.2 ml ethanol with sonication to remove *unreacted* ions at the final stage of preparation, followed by a final resuspension in water.

Spectroscopic measurements

Absorption spectra were measured on a Hewlett-Packard model 8543 spectrophotometer using 1-cm cuvettes. Steady-state fluorescence emission measurements were recorded using a Varian Eclipse spectrofluorometer. Transmission electron micrographs (TEM) were taken with a side-entry electron microscope (Jeol Jem 1200 Ex II Microscope). Samples were cast from water solutions onto standard carbon-coated (200–300 Å) Formvar films on copper grids (200 mesh) by placing a droplet of a ca. 1 mg/mL aqueous

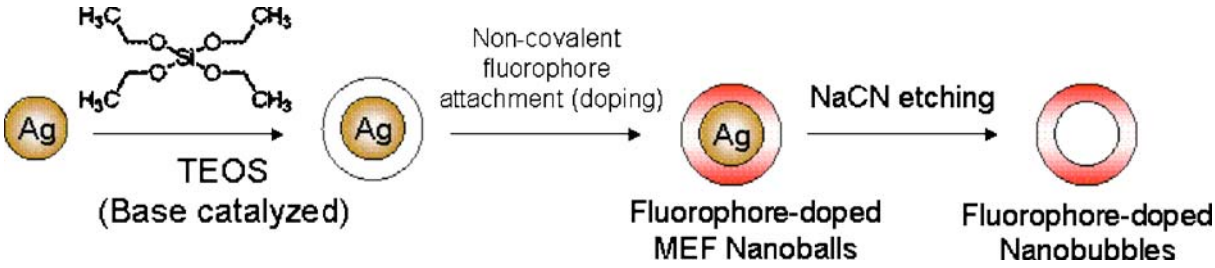

Fig. 1 Schematic for the preparation of fluorescent core-shell Ag@SiO_2 (MEF) nanoballs and fluorescent nanobubbles

sample solution on a grid, waiting 5 min, and removing excess solution by touching a small piece of filter paper to the edge of the grid. The grid was dried in air for 24 h. In some cases, ethanol solutions of the colloids were used and only 3 h was needed for drying.

Results and discussion

The preparation of fluorescent core-shell Ag@SiO_2 nanoballs (MEF nanoballs) was undertaken in three steps (Fig. 1): 1) firstly, silver colloids are prepared by reduction of silver nitrate by sodium citrate, 2) then a silica shell of various thickness was grown around the colloids, and 3) lastly, fluorophore (Rh800) was doped into the silica shell. In order to show the benefits of using a silver core in the MEF nanoballs, we have prepared fluorescent control probes *without* the silver core. The control fluorescent probes (without the silver core), named fluorescent n*anobubbles,* are prepared by dissolving the silver core away (etching) with cyanide from the fluorescent MEF nanoballs, c.f. Fig. 1. This procedure provided us with the best possible fluorescent control probes to evaluate the benefits of using a silver core: since the Rh800 employed here is hydrophobic [11] and retained in the hydrophobic pockets of the silica shell, the etching of the silver core with cyanide did not cause the removal of fluorophore from the shell (thickness $\approx$10 nm). Thus, it is possible to compare the fluorescence emission of the fluorescent MEF nanoballs and of the fluorescent nanobubbles in a *quantitative* manner.

We have prepared several MEF nanoballs with different thickness of the SiO_2 coating. The diameter of the silver core was 130 $\pm$ 10 nm for all the preparations (Fig. 2-inset), a size which has been shown most suitable for MEF and the Radiating Plasmon Model [12]. The thickness of the silica shell was varied from 2 to 35 $\pm$ 1 nm, to optimize fluorescence enhancement (data not shown) and was controlled by the concentration of TEOS after alkaline initiation (See Section 2.2.Methods).

Figure 2 shows the absorption spectra of two MEF nanoballs with different shell thicknesses. The surface plasmon resonance (SPR) peak of the silver shifted towards longer wavelengths as the thickness of the silica shell increased, as expected, and indeed observed by others [13]. The SPR peak of the MEF nanoballs with 2 and 35 nm shell thickness were observed at 437 and 479 nm, respectively. The nanobubbles however, show no plasmon absorption band, as the silver has been etched away by cyanide (data not shown). The importance of using the silica shell around the silver core is 3-fold: 1) silica layers offer the robustness, chemical inertness and the versatility needed for the conjugation of biomolecules or fluorophores; 2) it protects the silver core from ions present in biological media and 3) it allows for the distance dependent MEF phenomenon, which we have determined optimum for shell thicknesses $<$ 11 nm [1]. This thickness value is also consistent with values obtained from planar surfaces [1–10].

Figure 3 shows the fluorescence emission intensity from Rh800-doped MEF nanoballs and from the corresponding fluorescent nanobubbles (control samples) Rh800-doped nanobubbles. The emission intensity was approximately *20-fold* higher for Rh800-doped MEF nanoballs than Rh800-doped nanobubbles. The fluorescence emission spectra of the fluorophores were identical in both cases, indicating that the spectral properties of the fluorophores were retained.

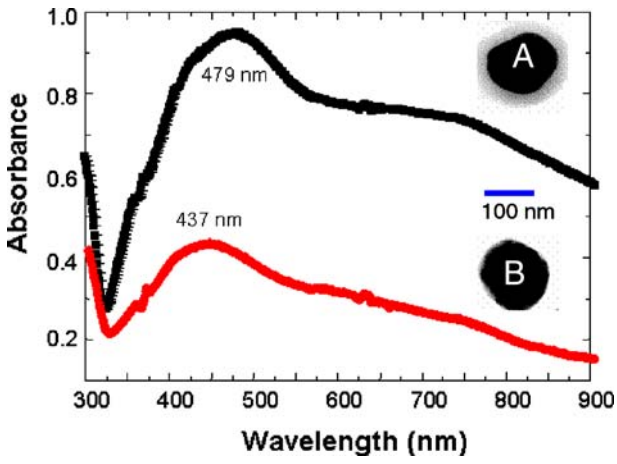

Fig. 2 Absorption spectra of two core-shell Ag@SiO_2 nanoballs with different shell thicknesses. Inset-TEM images of Ag@SiO_2 nanoballs. The diameter of the Ag is $\approx$130 $\pm$ 10 nm and the thickness of the shell is A = 35 $\pm$ 1 nm, B = 2 $\pm$ 1 nm. The scale bar is obtained from the TEM images

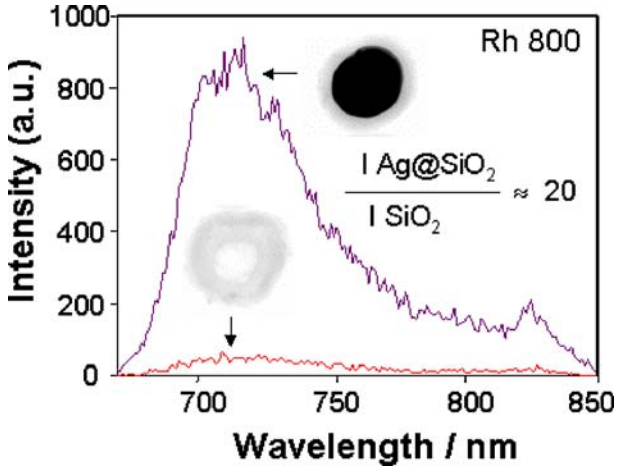

Fig. 3 Fluorescence emission spectrum of Rh800-doped MEF nanoballs and from the corresponding fluorescent nanobubbles (control sample, cyanide etched), Rh800-doped nanobubbles. The thickness of the shell is 11 ± 1 nm (optimized with respect to maximum emission intensity) for all the samples

It is well established that the interactions of fluorophores with silver nanoparticles results in fluorescence enhancement, an increased photostability, and a decreased lifetime due to increased rates of system radiative decay [1–12]. Although not shown here, the lifetime of Rh800 is significantly reduced, up to a 10-fold reduction in amplitude weighted lifetime is observed [1, 4]. The reduction in lifetime, in addition to an increase in fluorescence emission is particularly interesting for fluorescence based applications: where a 20-fold increase in intensity coupled with a 10-fold reduction in fluorophore-particle lifetime, provides for an ≈200-fold potential increase in overall particle detectability. In addition, a reduced lifetime, affords for increased fluorophore photostability [1], as there is less time for excited state photodestructive processes to occur such as oxidation, interaction with singlet oxygen or even superoxide. A detailed study of the MEF nanoballs, including lifetime analysis and their application for single nanoparticle detection is currently underway, and will be reported in due course.

Conclusions

In this Rapid Communication, we report the development of fluorescent core-shell $Ag@SiO_2$ nanoballs or MEF nanoballs for potential applications in Metal-Enhanced Fluorescence solution-based sensing. The diameter of the silver core of the MEF nanoballs was ≈130 ± 10 nm, while the thickness of shell was controllably varied between ≈2 and ≈35 nm. A model near infrared fluorophore, Rhodamine 800 (Rh800), was embedded into the shell to show the possible utility for cellular imaging. In order to show the benefits of using a silver core, we have prepared control samples (nanobubbles), by removing the silver core from the MEF nanoballs by chemical etching and subsequently compared the fluorescence emission from the MEF nanoballs and nanobubbles. The emission intensity from the MEF nanoballs was ≈20-fold larger than that of the nanobubbles. Given that the lifetime of the fluorophores decreases within close proximity to the silver surfaces [1–12], then this potentially allows for even better particle detectability as the fluorophore (particle) can be cycled at a higher rate, i.e more excitation/emission event cycles. A decreased lifetime combined with an increased fluorescence emission cumulatively provides researchers with a greater detectability and thus better sensitivity. We speculate that MEF nanoballs are likely to enhance the capability of solution-based MEF sensing platforms and predict their potential applications in a variety of biological applications, such as cellular entry, imaging and biological localization.

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