Immunoassay microarrays based on microcontact printing of proteins and fluorescent Eu:Gd_2O_3 nanoparticles as novel labels

Mikaela Nichkova [1], Dosi Dosev [2], Shirley J. Gee [1], Bruce D. Hammock [1] and Ian M. Kennedy [2]

[1]Department of Entomology, University of California Davis, mnichkova@ucdavis.edu ; [2] Department of Mechanical and Aeronautical Engineering, University of California Davis

Abstract

Protein microarrays have the potential to play a fundamental role in the miniaturization of biosensors, clinical immunological assays, and protein-protein interaction studies. Lanthanide oxide nanoparticles are attractive fluorescent labels in biochemistry because of their large Stokes shift, sharp spectra, long lifetime and lack of photobleaching. Here we present the application of fluorescent europium-doped gadolinium oxide (Eu:Gd_2O_3) nanoparticles as labels in immunoassay microarrays. The nanoparticles synthesized by spray pyrolysis were coated with the target molecule. Microarrays of antibodies were fabricated by microcontact printing in line patterns onto glass substrates and immunoassays were successfully performed using the corresponding functionalized nanoparticles. The applicability of the fluorophore nanoparticles as reporters for detection of antibody-antigen interactions has been demonstrated for two types of immunoassays: rabbit IgG/anti-rabbit IgG and phenoxybenzoic acid (PBA)/anti-PBA IgG. The developed immunoassays were characterized by fluorescence microscopy and scanning electron microscopy. This works suggests that the use of the nanoparticles in multianalyte immunodiagnostics is very advantageous.

Properties of the Eu:Gd_2O_3 nanoparticles

Fluorescent Eu^{3+}-doped gadolinium oxide nanoparticles are synthesized by **spray pyrolysis**. This method is fast, simple, efficient and inexpensive.

Under properly controlled conditions the flame-synthesized nanoparticles have **spherical shape**. A narrow size range (5 – 100 nm) can be obtained by centrifugal settling.

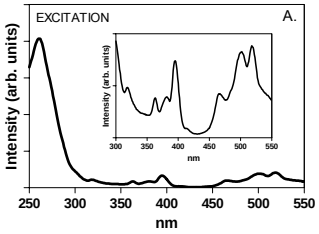


Figure 1. **Time-resolved fluorescence spectra** of Eu:Gd_2O_3 nanoparticles. Excitation spectra at 615 nm (**A**) and emission spectra at 260 nm (**B**). The half-width of the emission peak is 5 nm which is very narrow in comparison with other fluorophores. Fluorescence lifetime is about **1 msec**.

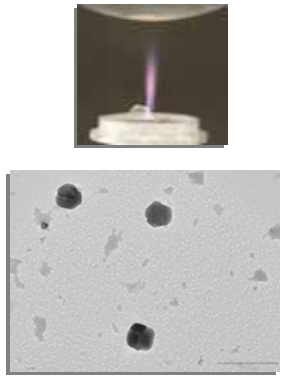


Figure 2. **Transmission electron microscope** image of **100 nm** Eu:Gd_2O_3 nanoparticles obtained by spray pyrolysis.

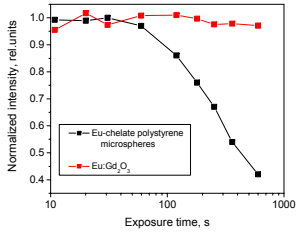


Figure 3. **Photostability** of Eu:Gd_2O_3 nanoparticles and commercially available Eu-chelate polystyrene microspheres. Exposure to pulsed 10 ns UV laser with energy 20-30 μJ, at frequency 20 Hz.

Good photostability of Eu:Gd_2O_3 makes them suitable biolabel for fluorescence microscopy

Introduction

Emerging techniques in biochemistry and biosensor development are based on protein and DNA microarrays formed by micro contact printing[1,2]. Microarrays are usually visualized using organic fluorescent dyes[3]. However, the poor photostability and brightness, especially for samples with high background fluorescence, limit the effectiveness of these fluorophores in microarrays applications. New fluorophores (quantum dots[4], dye-doped silica nanopartilces[5]) has been used recently for imaging of DNA microarrays. As an alternative, the unique properties of nanoparticles made from lanthanide oxides (long fluorescence lifetime, emission independent of the size, simple production) make them promising for low-cost applications in biochemistry. Preliminary results using europium oxide (Eu_2O_3) nanoparticles as labels for environmental immunoassay have already been reported showing enhanced assay sensitivity[6].

Objective

To apply novel fluorophores (Eu^{3+}- doped gadolinium oxide nanoparticles) to the imaging of protein microarrays prepared by μCP.

Biochemical functionalization of the nanoparticles

A variety of proteins (**IgG, avidin, BSA, protein A**) adsorb spontaneously on the surface of the nanoparticles. This coating method is an easy one step procedure yielding conjugates stable in the most commonly used buffers. The fluorescence of the nanoparticles is not affected by the protein layer. The adsorbed proteins retain their activity. The number of binding sites on the surface can be controlled by co-adsorption with BSA or a non-specific antibody. The surface of the nanoparticles can be efficiently blocked with BSA to avoid non-specific binding in immunoassays

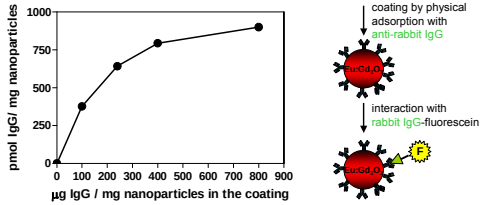
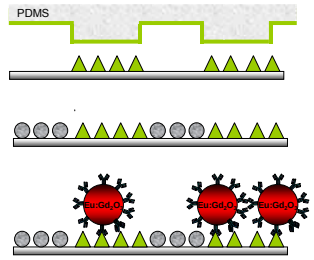


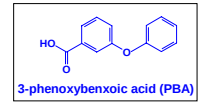
Figure 4. **Determination of active binding sites** on the surface of Eu:Gd_2O_3 nanoparticles coated with anti-rabbit IgG using rabbit IgG labeled with fluorescein and measuring the fluorescein fluorescence from the particle-bound labeled rabbit IgG.

Immunoassay microarrays



► **Microcontact printing** of the protein (**rabbit IgG** or the antigen **BSA-PBA**) onto glass substrate using polydimethylsiloxane (PDMS) stamp (10 x 10 μm)

► **Blocking** the non-printed glass surface with BSA



► **Immunoreaction** between the **anti-rabbit IgG** (or **anti-PBA-IgG**) coated Eu:Gd_2O_3 nanoparticles

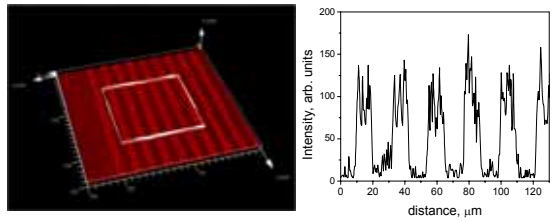


Figure 5. **Confocal microscope fluorescent image** of specifically bound **anti-PBA IgG** coated Eu:Gd_2O_3 nanoparticles to the microcontact-printed **BSA-PBA** antigen. The non-printed surface is blocked with BSA (left). Fluorescence intensity profile of the micropatterns corresponding to the same image (right).

The **specific binding** reaction between the nanoparticle-labeled IgG and the printed antigen generate fluorescent strips with nearly uniform intensity. The bound nanoparticles are arranged into an almost perfect match of the printed pattern (10 μm width).

The **lack of photo-bleaching** of the Eu:Gd_2O_3 nanoparticles allowed the fluorescent image to be observed for unlimited period of time providing the possibility for image optimization.

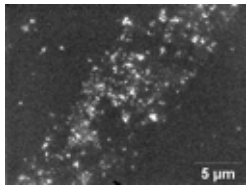


Figure 6. **Scaning electron microscopy** image of specifically bound **anti-PBA IgG** coated Eu:Gd_2O_3 nanoparticles to microcontact printed **BSA-PBA** antigen.

The presence of specifically bound nanoparticles is confirmed with SEM. The smallest particles that can be observed individually are about 100 nm. Larger and brighter spots represent areas of local higher density. Only few individual particles can be observed at the non-printed (blocked) area between the strips that shows **very low non-specific binding**.

Conclusions

► **Fluorescent nanoparticles made of lanthanide oxides can be successfully used for imaging of protein micropatterns**. Their photo stability gives unlimited time for image observation and optimization. The approach is succesufully applied to micropatterns formed by proteins (IgG) and small molecules (haptens, ex. PBA).

► The surface properties of the Eu:Gd_2O3 nanoparticles permit easy **one-step biofunctionalization** with the desired protein. Avidin – coated nanoparticles can be used as a base shell for the preparation of conjugates with a variety of biotinylated antibodies and DNA. Direct coating with functional antibodies controlling the number of binding sites on the nanoparticle surface is performed too. The strong adsorption of BSA to the nanoparticle surface can be used as an easy way to functionalize the partcles with small molecules (biotin, haptens).

►The methodology developed in this work can be easily applied to lanthanide doped nanoparticles with different fluorescent emission (Tb, Sm, Dy) allowing **multi-analyte detection.** The fluorescent nanopartciles can be used as suitable **labels in protein and DNA microarrays formats**.

References

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