Immunoassay microarrays based on microcontact printing of proteins and fluorescent Eu:Gd$_2$O$_3$ nanoparticles as novel labels

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

1Department 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 bioassays, 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$_2$O$_3$) 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. 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$_2$O$_3$) nanoparticles as labels for environmental immunoassays have already been reported showing enhanced assay sensitivity.

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, dye-doped silica nanoparticles 4) 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$_2$O$_3$) nanoparticles as labels for environmental immunoassays have already been reported showing enhanced assay sensitivity.

Objective

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

Properties of the Eu:Gd$_2$O$_3$ 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.

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 for non-specific affinity. The surface of the nanoparticles can be efficiently blocked with BSA to avoid non-specific binding in immunoassays.

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 successfully applied to micropatterns formed by proteins (IgG) and small molecules (haptens, ex. PBA).

The surface properties of the Eu:Gd$_2$O$_3$ 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 particles 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 nanoparticles can be used as suitable labels in protein and DNA microarrays formats.

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