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Optimization of antibody-conjugated magnetic nanoparticles for target preconcentration and immunoassays

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ABSTRACT

Biosensors based on antibody recognition have a wide range of monitoring applications that apply to clinical, environmental, homeland security, and food problems. In an effort to improve the limit of detection of the Naval Research Laboratory (NRL) Array Biosensor, magnetic nanoparticles (MNPs) were designed and tested using a fluorescence-based array biosensor. The MNPs were coated with the fluorescently labeled protein, AlexaFluor647–chicken IgG (Alexa647–chick IgG). Antibody-labeled MNPs (Alexa647–chick–MNPs) were used to preconcentrate the target via magnetic separation and as the tracer to demonstrate binding to slides modified with anti-chicken IgG as a capture agent. A full optimization study of the antibody-modified MNPs and their use in the biosensor was performed. This investigation looked at the Alexa647–chick–MNP composition, MNP surface modifications, target preconcentration conditions, and the effect that magnetic extraction has on the Alexa647–chick–MNP binding with the array surface. The results demonstrate the impact of magnetic extraction using the MNPs labeled with fluorescent proteins both for target preconcentration and for subsequent integration into immunoassays performed under flow conditions for enhanced signal generation.

Biosensors are under development for target screening in clinical, environmental, water, and food samples [1–4]. An essential component of these systems is the recognition elements, often antibodies, for selective identification of target analytes. Antibodies have demonstrated high binding affinities with extraordinary specificity for target molecules even in complex sample matrices and with low target concentrations [5]. The Array Biosensor developed at the Naval Research Laboratory (NRL),[2] which typically performs multiplexed immunoassays, has been used successfully for the detection of a variety of proteins, molecules, viruses, and bacteria in complex sample matrices [6,7]. The two-dimensional nature of the sensing surface facilitates simultaneous analysis of multiple samples for multiple analytes. The immunoassays developed to date are rapid (15–25 min) and automated, with little or no sample pretreatment prior to analysis [8]. Limits of detection (LOD) obtained with the NRL Array Biosensor are comparable to other rapid biosensor technologies and enzyme-linked immunosorbent assays (ELISAs). However, the NRL system falls short of the LODs desired for some targets, particularly bacterial species, compared with those obtained by the more time-consuming and complex "gold standard" methodologies such as cell culture and polymerase chain reaction (PCR). To overcome this limitation, one approach would be to include a target preconcentration step prior to the immunoassay. However, to keep the detection method practical, any sample treatment steps must be simple to perform, add minimal time to the analysis, and improve the overall assay results.

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[2] *Abbreviations used:* NRL, Naval Research Laboratory; LOD, limit of detection; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; MP, magnetic particle; HSA, human serum albumin; MNP, magnetic nanoparticle; MATEFF, magnetically assisted transport evanescent field fluoroimmunoassay; Alexa647–chick–MNPs, MNPs functionalized with fluorescently labeled target chicken IgG with AlexaFluor647; MTS, 3-mercaptopropyl trimethoxy silane; GMBS, *N*-(γ-maleimidobutyryloxy) succinimide ester; TEOS, tetraethylorthosilicate; Mes, 2-(*N*-morpholino) ethane sulfonic acid; PDMS, poly(dimethyl) siloxane; carboxyl–silane, carboxyethylsilanetriol sodium salt; EDC, 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride; NHS, *N*-hydroxysuccinimide; Rb–anti-chick IgG, rabbit anti-chicken IgY; PBS, phosphate-buffered saline; UV–Vis, ultraviolet–visible; PEG, polyethylene glycol; BSA, bovine serum albumin; PBST, PBS + 0.05% Tween; PBSCD, PBS/0.1% casein/0.05% deoxycholic acid; CCD, charge-coupled device; DOC, deoxycholic acid.

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Immunomagnetic separation (IMS) is one preconcentration technique that is commonly used prior to detection for sample preparation and cleanup. Magnetic particles (MPs) are becoming increasingly popular for automated separations [9,10]. These magnetic materials are easily manipulated using magnetic fields and are removed from solutions in a matter of minutes. With surface modification, MPs have been labeled with a variety of biological molecules that have the ability to scavenge for targets of interest and separate them from complex biological media, potentially improving the LOD of subsequent analysis techniques. Commercially available MPs are typically 0.5 to 2 μm in diameter and come with a variety of chemically active surfaces that can be used to functionalize the particle with the desired capture agent, offering a large surface area for target capture.

Common formats for quantification of targets collected by MPs are typically independent of the particles themselves. Such methods include culture, flow cytometry analysis [11], PCR coupled with hybridization [12], electrochemical measurements [13,14], and ELISAs [15–17]. When fluorescence species are added, quantification of the resulting fluorescent immunomagnetic–target complex is normally achieved using devices such as a spectrometer [18,19], a flow cytometer [11,20], or a fluorescence microscope [21,22]. Increasingly, researchers are using the properties of the MPs themselves to determine the presence of the bound target [23,24] with devices such as giant magnetoresistive (GMR) sensors [25,26], the superconducting quantum interference device (SQUID) [27], and the magnetic permeability-based assay [28]. Interestingly, Colombo and coworkers [29] recently used the proton T_2 relaxation time of water molecules surrounding human serum albumin (HSA)-modified magnetic nanoparticles (MNPs) as a sensor for anti-HSA detection.

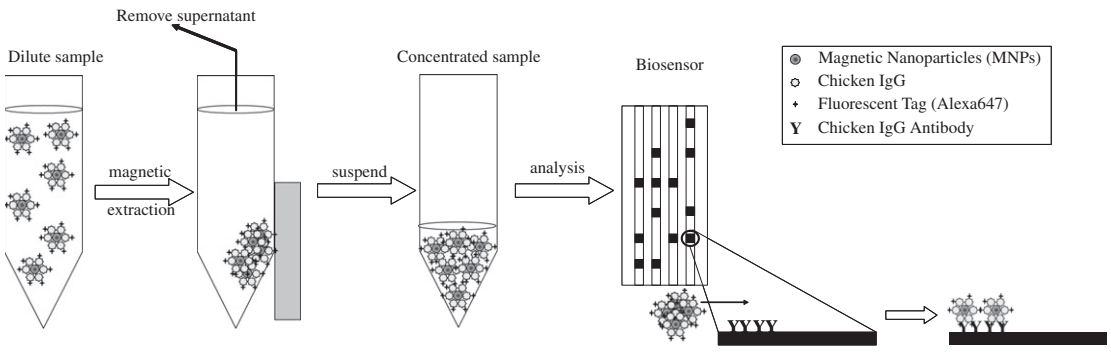
Advances in microfluidics and integrated technologies have resulted in the use of MPs coupled with planar surfaces [15,16,24–26]. Wellman and Sepaniak [30] demonstrated that magnetic beads functionalized with a fluorescence antibody complex could be transported, using an external magnetic field, into the region of an evanescent field for detection, a technique referred to as magnetically assisted transport evanescent field fluoroimmunoassay (MATEFF). Morozov and Morozova [31] investigated a number of methods for interacting antibody-labeled MPs with protein microarrays, including a magnetic brushing technique, magnetic scanning, and a push/pull method that used a magnet below the substrate to concentrate the beads on the surface and a second magnet above the substrate to remove weakly bound or nonspecifically bound MPs [31]. They recently extended their studies to look more closely at force differentiation and shear stress under flow [32]. These studies use complicated schemes to facilitate MP interaction with the surface, in large part due to the relatively large sizes of the commercial MPs used. The binding of large antigen–antibody–MP complexes to an antibody immobilized on a sensor surface is subject to shear and subsequent dissociation from the surface in the flow conditions normally used in such immunoassays [32]. One way to address this problem would be to decrease the size of the MPs used. Nano-sized magnetite particles enveloped in lipid membranes, produced by bacteria, have been used in a number of studies [33]. Modifiable iron oxide-based MNPs have also been synthesized with a well-defined size and shape [34,35]. MNPs are being used in ELISAs [15–17], lateral flow immunoassays [36], and a magnetic force microscopy bioassay for biotin–streptavidin [37] and IgG detection [38]. Although Morozov and Morozova [31,32] have studied micron-sized MPs interacting with protein microarrays, to date there are limited studies that use nano-sized MPs to facilitate both target concentration and signaling events for immunoassays [36–38].

In this study, fluorescently tagged antibodies attached to MNPs were employed in a simple target preconcentration step. The extracted target–antibody–MNPs were introduced directly to the Array Biosensor under flow conditions to initiate signal transduction, and the effect of the target preconcentration and nanoparticle-based fluorescence signal generation was evaluated. This method was used to improve the overall LOD of the Array Biosensor (Scheme 1). Unlike the previously mentioned MATEFFs, these MPs are not used simply to localize the target to the evanescent field sensing surface but rather to simultaneously perform both a target concentration and a signaling function on the microarray. For optimization purposes, a simple direct binding assay was investigated. MNPs were functionalized with the fluorescently labeled target chicken IgG with AlexaFluor647 (Alexa647–chick–MNPs). The sensor surface was patterned with rabbit–anti-chicken IgG. The assay was used to evaluate the surface composition of the modified MNPs prepared under a variety of conjugation conditions, the extraction time for preconcentration experiments, the sample concentration achieved by magnetic extraction, and the effect of magnetic extraction on the MNP on particle aggregation and binding. The final signals were produced as the result of the binding of the MNPs to the slide surface.

Materials and methods

Materials

Unless otherwise specified, chemicals were of reagent grade and used as received. All chemicals, including 3-mercaptopropyl trimethoxy silane (MTS), *N*-(γ-maleimidobutyryloxy) succinimide ester (GMBS), tetraethylorthosilicate (TEOS), and 2-(*N*-morpholino) ethane sulfonic acid (Mes), were purchased from Sigma–Aldrich (St. Louis, MO, USA) unless otherwise noted. Poly(dimethyl) siloxane (PDMS), used for making the assay flow cells, was obtained from Nusil Silicone Technology (Carpinteria, CA, USA). Borosilicate glass slides from Daigger (Vernon Hills, IL, USA) were used in all of the



Scheme 1. Magnetic extraction, sample concentration, and analysis. The MNPs modified with fluorescently tagged antibody are extracted from the large volume sample, the extracted MNPs are resuspended in 0.2 ml of buffer, and analysis is performed using the Array Biosensor with the MNPs functioning as the tracer.