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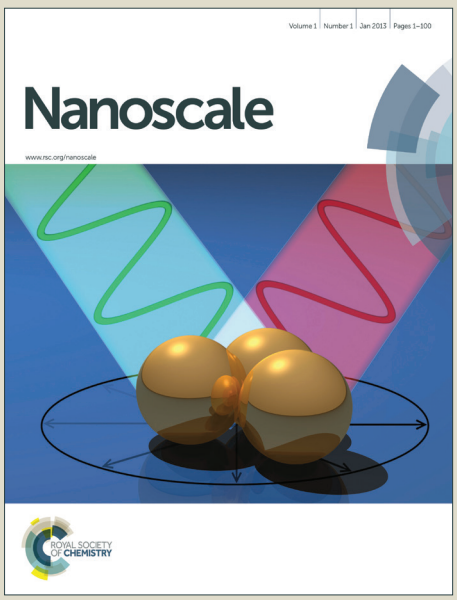

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ARTICLE

Magnetic bead-quantum dot assay for detection of a biomarker for traumatic brain injury

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Abstract Current diagnostic methods for traumatic brain injury (TBI), which accounts for 15% of all emergency room visits, are limited to neuroimaging modalities. The challenges of accurate diagnosis and monitoring of TBI have created the need for a simple and sensitive blood test to detect brain-specific biomarkers. Here we report on an assay for detection of S100B, a putative biomarker for TBI, using antibody-conjugated magnetic beads for capture of the protein, and antibody-conjugated quantum dots for optical detection. From Western Blot, we show efficient antigen capture and concentration by the magnetic beads. Using magnetic bead capture and quantum dot detection in serum samples, we show a wide detection range and detection limit below the clinical cut-off level.

Introduction

Traumatic brain injury (TBI) results from events such as a rapid change in motion, a direct impact to the head, or an explosive blast, and is a major health problem affecting nearly 2 million people in US every year.[1-3] The two main diagnostic methods for TBI, depending on the patient's symptoms, are clinical assessment, e.g. Glasgow Coma Scale, and neuroimaging techniques such as CT and MRI.[1-3] Mild TBI (mTBI), which accounts for more than 90% of TBI cases, is associated with diffuse brain damage and non-specific symptoms, and hence represents a major diagnostic challenge.[1-3] These challenges have created a need for an alternative method.

Current research in biomarkers for TBI provides the potential for the development of non-invasive diagnostics, such as a blood test.[1-5] Biomarkers such as RNA, metabolites, lipids, peptides, proteins, or autoantibodies against proteins released from the diseased/injured state can be used to objectively diagnose mTBI.[1-5] Following injury, protein biomarkers penetrate the blood-brain barrier (BBB) and are released into the bloodstream, cerebrospinal fluid, or other body fluids. Many clinical studies have shown that TBI biomarker levels are elevated within 24 hours of injury.[1, 2]

The most extensively studied biomarker for mTBI is S100B. S100B is a brain-enriched member of the S-100 family of low molecular weight (10.5 kDa) calcium-binding proteins.[1, 6-8] S100B is predominantly expressed in astrocytes, constituting 1 - 1.5 µg/mg of all soluble protein in the brain.[2, 6, 7] S100B can be found in very low levels in human cerebrospinal fluid (CSF) and serum (<0.25 ng/mL). Many clinical studies have reported elevated S100B levels in the serum of patients with traumatic head injury,[1, 6-8] Although some studies have not found a correlation,[9, 10], S100B is still being investigated as a prognostic marker for traumatic brain injury.[11] However, the main limitation for the use of biomarkers for TBI is that these concentrations are below the lower limit of detection by most standard immunoassays. Therefore, there is a critical need to develop a sensitive assay for reliable quantification of these low abundance proteins.

The immunoassay commonly used to determine protein concentration in serum is the commercial enzyme-linked immunosorbent assay (ELISA) kit. Although the ELISA method is considered accurate and reliable, the assay is time-consuming with a narrow linear range and short enzyme/substrate reaction time.[12]

The two key steps in any immunoassay are capture and detection. Magnetic beads (MBs) are widely used for biomedical applications such as extraction or purification of biomolecules such as proteins, antibodies, and nucleic acids.[13-16] Bio-functionalized MBs are often used in cell sorting, bioseparation, targeted drug delivery, and immunoassays.[17-21] MBs functionalized with targeting moieties such as antibodies enable efficient collection, separation, and recognition of target molecules in a simple and rapid process without any centrifugation or filtration. Importantly, MBs have a high surface-to-volume ratio and in suspension can result in short diffusion lengths, important for the capture of low abundance analytes.

QDs are inorganic semiconducting nanoparticles with unique optical properties, such as broad absorption spectra, narrow

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emission spectra, photo-bleaching stability, and brightness. Various functionalization and bioconjugation strategies have been developed to enable QD labeling and detection of various biological analytes, including small molecules, proteins, cells, and bacteria. We have previously used QDs for quantitative profiling of cell surface membrane biomarkers.[22, 23] Recently, surface functionalized QDs in conjunction with magnetic beads have been used to label captured analytes through antibody-analyte binding as signal transduction probes.[12, 24-32]

Here we report on a magnetic bead – quantum dot (MB-QD) sandwich assay for capture and detection of S100B in serum (Figure 1). Magnetic beads enable fast capture, concentration, magnetic separation, and washing. Sandwich binding of two monoclonal antibodies yields high specificity, and QD fluorescence detection provides high sensitivity. Moreover, by releasing the QDs following binding eliminates the interference of magnetic beads in signal detection and enhances the detection resolution of the assay. Our objectives are: (1) to develop a relatively fast and sensitive assay, (2) to provide a reliable method to validate antibody/antigen affinity, (3) to demonstrate capture and concentration of the target antigen, (4) to provide an efficient method to conjugate antibodies to QDs without adding additional chemical reagents and purifications steps, (5) to further enhance the detection resolution of the assay by releasing QDs from the immunocomplex, and (6) to demonstrate a wide dynamic range and detection limit below the clinical cut-off value for S100B.

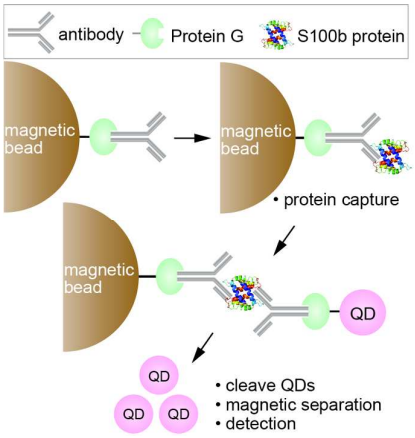

Figure 1. Schematic illustration of the MB-QD sandwich assay and release of QDs. Magnetic beads are conjugated with an antibody for capture of the target protein. A protein G linker allows conjugation of any IgG antibody without changing the surface functionalization. Typical antibody concentrations are 8 µg per mg of MBs. Following incubation with the test solution and capture of the target protein, the MBs are incubated with QDs functionalized with a complementary antibody using protein G for coupling. Following washing, the QDs are cleaved from the MBs for optical detection.

Experimental

Antibody conjugation of magnetic beads (MBs). Recombinant protein G (17 kDa; 10004D, Life Technologies) was covalently coupled to the surface of superparamagnetic magnetic beads (Dynabeads, Invitrogen). 3 mg of MBs were washed with PBST (PBS, 0.1% tween 20) three times, magnetically separated, and re-suspended in 400 µL of PBST. 24 µg of antibodies (Capturing antibody: monoclonal anti-S100B, clone 1B2 and detection antibody: monoclonal anti-S100B, clone 2A10, Abnova) were added to the MBs and incubated in room temperature for 30 minutes. MB-Ab conjugates were washed with PBST five times and re-suspended in 2000 µL of PBST.

Protein capture, SDS-PAGE, and Western blotting. Human S100 beta full length protein (ab55570, Abcam) was constituted at 1 µg mL^{-1} in sterile PBS. 100ng, 10ng, and 0 ng of S100B in 100 µL of normal human serum (S1-100ML, EMD Millipore) were placed in conical tubes (Protein Lobind tubes, Eppendorf®), each containing 100 µL of MB-Ab conjugate (150 µg). Tubes were placed on a rotator for 3 hours in room temperature for the protein capture. The MB-Ab-S100B complexes were then washed with PBST three times for 5 minutes. The wash buffer was removed with a pipette after magnetic separation.

Next, 10 µL of antibody elution buffer (Fisher, PI21004), 5 µL of NuPAGE® SDS sample buffer (Life Technologies, NP0007), and 3 µL of NuPAGE® sample reducing agent (Life Technologies, NP0004) were added to each tube containing MB-Ab-S100B complexes. Samples were vortexed and placed in a water bath at 89˚C for 15 minutes, and vortexed again for 30 seconds. The tubes were then centrifuged for 10 seconds at 3,000xg and the prepared samples added to each lane of an 8 - 16% precast gel (Bio-Rad, 4561104). SDS-PAGE was performed in MOPS running buffer at 180 V. The gel was then transferred to a nitrocellulose membrane (Bio-Rad, 1704158) using a Trans-blot Turbo Transfer instrument. The membrane was blocked with milk (5% powdered milk in TBS, 0.1% Tween 20) for 1 hour. Anti-S100 (polyclonal rabbit antibody, Dako, Z031101-2) was used at 1:1000 dilution in 5% milk to detect proteins. Secondary anti-rabbit HRP conjugate was used for visualization of the blots.

QD synthesis and lipid functionalization. CdSe cores were synthesized from CdO and Se in TOPO and HDA and passivated with a (Cd,Zn)S shell, as described previously.[33] The core/shell QDs have an average diameter of 8 nm and an emission peak at about 605 nm.[33] The quantum yield is about 60%.[22,33] The concentration of the QDs in chloroform was determined from the absorbance at 350 nm using Beer's Law (A = εlc) and an extinction coefficient $\varepsilon = 1.44 \times 10^{26} \times r^3$ (cm^2 mol^{-1}). After washing in hexane, the QDs were precipitated with methanol, dried under vacuum, and re-suspended in 4 mL of chloroform at a concentration 30 to 40 mg mL^{-1}.

The QDs in chloroform were water solubilized by forming an outer lipid layer consisting of a single acyl chain lipid to accommodate the high curvature, a double acyl chain phospholipid with a terminal PEG group for stability, and a double acyl chain phospholipid with a terminal succinimidyl ester for antibody conjugation group.[22, 23, 33] 0.5 nmoles of QDs and a 7-fold excess of lipids in chloroform were mixed and the chloroform is evaporated off using nitrogen gas. 1 mL of water (pH 5.5) was added and vortexed until the solution turns clear.

The resulting solution was passed through a syringe filter with a 100-nm polytetrafluoroethylene membrane to remove any aggregates.[33] For all experiments reported here the lipids consisted of 80 mol% MHPC (1-myristoyl-2-hydroxy-sn-glycero-3-phosphocholine) (Avanti Polar Lipids, Inc, Alabama), 10 mol% DSPE-PEG2K (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000])(Avanti Polar Lipids, Inc, Alabama), and 10 mol% DSPE-PEG3K-NHS ester (N-hydroxysuccinimide ester) (Nanocs, Inc, New York). The excess was calculated based on a diameter of the QDs with a TOPO/HDA inner leaflet of 14 nm, an average footprint of 0.7 nm^2 for the double acyl chain DSPE lipids, and an average footprint of 0.6 nm^2 for MHPC.[34-36] Assuming that the lipid composition in the outer leaflet on the QDs is the same as in bulk solution, then each QD has 821 MHPC molecules, 88 DSPE-PEG2k, and 88 DSPE-PEG3K-NHS ester.

Protein G and antibody conjugation to QD. Lipid coated QDs were incubated with Protein G (Abcam, ab155724), constituted at 1 mg mL^{-1} in sterile PBS, in 1:5 molar ratios, and PBS (10x, pH 7.4, Gibco®) was used to adjust solution to pH 7.4. The reaction was performed at room temperature for 1 hour. The resulting solution was passed through a syringe filter with a 100-nm polytetrafluoroethylene membrane to remove any aggregates. Next, 0.1% BSA (Sigma, 5470) was added and stored in 4˚C. The detection antibody, monoclonal anti-S100B (clone 2A10, Abnova), as supplied (100 µg in 200 µL) was added to a suspension of the QD-Protein G complex at a concentration to give an average of three antibodies per QD, and incubated for 1 h at RT.

Sandwich Assay. MB-Ab conjugates were used to capture S100B in serum. QD-Ab conjugates were used for detection and quantification of the captured protein concentration (Figure 4A). Different concentrations of S100B (0, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1 and 3 ng) were spiked into 100 µL of normal human serum. Protein capture was performed at 37˚C for 15 minutes using 150 µg of MB-Ab conjugate for each sample. Next, 2 pmoles of prepared QD-Ab conjugates were added to each sample and incubated for 15 minutes. All steps were carried out on a tube rotator in a dry incubator at 37˚C. Unbound QDs were removed after magnetic separation. Samples were washed with PBST three times and re-suspended in PBS for fluorescence measurements.

QD elution from immunocomplexes. The elution buffer was prepared in 10 mM borate buffer (pH 8.5) with 8 M urea and 0.1% BSA. After the sandwich assay was completed, 200 µL of elution buffer was added to each sample and placed on a rotator for 20 minutes. Next, MBs were magnetically separated and the supernatant (eluted QDs) collected for fluorescence measurements.

Results and discussion

Universal antibody conjugation using Protein G. The conventional approach for conjugating antibodies to particles generally involves modification of the nanoparticle surface with groups that react with the side group of a surface residue of the antibody using various bioconjugation chemistries. These direct coupling methods usually result in a random orientation of the antibody on the nanoparticle surface such that the antigen binding sites may not be accessible.[37, 38] Non-covalent coupling methods have also been explored for antibody conjugation but the most promising involve functionalization of the nanoparticle with immunoglobulin binding proteins, such as protein A and protein G.[23, 39, 40] These adapter proteins contain Fc-binding domains and allow optimal antibody orientation.[23]

Antibody conjugation to the protein G modified superparamagnetic beads requires a simple and short incubation followed by a gentle separation and washing step that result in minimal physical stress to the antibodies (Figure 2A). Furthermore, the use of protein G for antibody coupling allows different antibodies to be used without changing the coupling protocol.

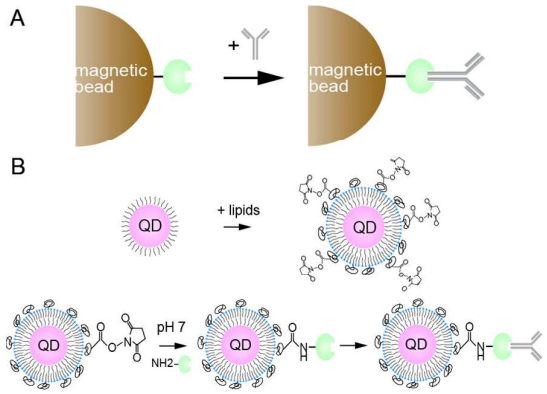

Figure 2. Functionalization of MBs and QDs. (A) The capture antibody is bound to the magnetic beads using protein G covalently coupled to the surface. (B) QD functionalization involves the formation of an outer lipid layer on the core/shell QDs with native hydrophobic surfactants. The lipid layer includes a single acyl chain lipid, a double acyl chain lipid with a PEG group, and a double acyl chain phospholipid with a PEG group and NHS ester moiety attached to the head group. Protein G with a primary amine crosslinks with NHS ester. The detection antibody is bound to the protein G.

QD functionalization. To conjugate protein G to lipid-encapsulated QDs, we used a succinimidyl-ester (NHS ester) motif (Figure 2B). NHS-esters are popular amine-specific functional groups that are incorporated into reagents for protein crosslinking and labeling.[41] For protein G conjugation the lipid layer on the QDs includes a double acyl chain phospholipid with a 3k PEG and an NHS ester terminal group (DSPE-PEG3k-NHS ester). Since NHS-ester moieties are moisture-sensitive, these lipids are equilibrated to room temperature before use and exposure to air minimized to avoid moisture condensation. The NHS ester groups on the QDs react with primary amine groups (-NH) of protein G to form stable amide bonds at pH 7 - 9. Hydrolysis of the NHS ester competes with the primary amine reaction.[41] The rate of hydrolysis increases with buffer pH and contributes to less efficient crosslinking. In order to minimize hydrolysis of the NHS ester, the lipid encapsulation of the QDs is performed in water at pH 5.5. Protein G is mixed with QDs immediately

after lipid encapsulation and concentrated PBS (amine-free) buffer is used to increase the pH to 7.4 for efficient crosslinking.

QDs coupled with protein G are then conjugated with the detection antibodies in 1:3 ratio. Our method of protein G and antibody conjugation to the QD is performed in physiological solution with no additional chemical reagents, thereby avoiding the necessity for additional purification steps and problems with antibody stability and functionality.

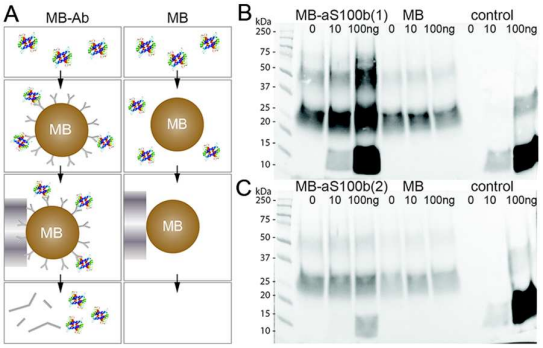

Figure 3. Antibody validation. (A) Immunomagnetic capture assay. Magnetic beads conjugated with and without antibodies incubated in serum containing S100B. After magnetic separation and washing, proteins bound to MBs are cleaved using a reducing reagent. Denatured protein collected after elution are loaded on to the gel for SDS-PAGE. Immunoblot analysis of (B) the capture antibody and (C) the detection antibody conjugated to MBs for capture of S100B spiked in normal human serum. Lanes 1-3 shows capturing of 0, 10, and 100 ng of S100B using MB-Abs. MBs without antibodies show no capture of antibodies (lanes 4-6). Bands at 50 kDa and 25 kDa are denatured heavy and light chains of antibodies (anti-S100B and endogenous antibodies in human serum). Lanes 7-9 shows 0, 10, and 100 ng of free S100B.

Antibody pair validation. For sandwich-type immunoassays, a matched pair of antibodies having an affinity to different epitopes on the antigen is required. For our MB-QD sandwich assay for S100B detection, eight different antibodies were screened and two were selected for capture and detection. In order to verify and evaluate the affinity of these selected antibodies, we performed a validation test by combining immunomagnetic capture (Figure 3A) and immunoblot analysis (Figure 3B,C). First, the magnetic beads were conjugated with an S100B antibody. MB-Ab conjugates were then incubated with normal human serum containing 0, 10, and 100 ng of S100B. After magnetic separation and several washing steps, all the protein bound to the MBs was cleaved using the elution buffer and reducing reagent dithiothreitol (DTT), and denatured for SDS-PAGE. Immunoblot results (Figure 3A) show three distinct bands at 50 kDa, 25 kDa, and 12 kDa. The first two bands correspond to heavy and light chains of the S100B antibodies (and any endogenous antibodies in the human serum) cleaved from the MBs. MBs coupled to the S100B antibodies used for capture (Figure 3B) and detection (Figure 3C) show an S100B band at 12 kDa, similar to the bands of free control protein with an intensity that is dependent on concentration. In contrast, MBs without antibodies show no protein capture (Figure 3B,C). This validation test shows that: (1) both capture and detection antibodies have affinity to S100B, and (2) that the protein G coupling is an efficient method of antibody conjugation. This also confirms that MBs have a capability of efficiently conjugating antibodies.

MB-QD sandwich assay. To demonstrate the complete sandwich assay (Figure 4A), magnetic beads conjugated with capture antibodies (MB-Abs) were incubated in 100 µL of normal human serum spiked with 1 pg to 3 ng of S100B. MB-Ab conjugates were also incubated in serum without S100B as a control. Following incubation with the QD-Ab conjugates, the fluorescence intensity of the control sample was used to determine the background signal to be subtracted from the measurements. (Figure 4C,D)

Here our goal is to demonstrate detection of S100B over the range of concentration required for diagnosis of mild traumatic brain injury. Many clinical studies have reported an S100B cut-off level for mild traumatic brain injury in the range from 0.1 to 0.25 ng mL^{-1}.[1, 2, 6, 7] For moderate to severe head injuries, the level of S100B in serum is reported to be in the range 5 - 20 ng mL^{-1}.[4, 5, 8] The concentration of many biomarkers for mTBI, including S100B, are below the lower limit of detection by most standard immunoassays.[1-3, 6]

The capture and detection steps were optimized for a target S100B concentration up to 3 ng (0.28 pmol) in 100 µL serum (30 ng mL^{-1}). Magnetic beads have a maximum binding capacity of 8 µg antibodies per mg of beads. To ensure that there is an excess of capture antibodies, 1.2 µg of antibodies (8 pmol) are conjugated to 150 µg of MBs. This gives a 40-fold excess of capturing antibodies to S100B at the highest concentration.

Following incubation of the MBs with S100B in human serum, 2 pmol of QD-Ab conjugates are added for detection. This gives approximately 10-fold excess of QD-Ab conjugates per S100B molecule for detection at the highest concentration. The detection range can be shifted to higher concentrations by increasing the number of MBs for capture and the number of QDs for detection.

After washing three times in PBST using magnetic separation, the sandwiched immunocomplex (MB-Ab-S100B-Ab-QD) was re-suspended in buffer. Following capture of the target protein, incubation with the QD-Ab conjugates, and separation to isolate the MB-Ab-S100B-Ab-QD conjugates, imaging for quantification can be performed in two ways: (1) QDs on the MBs, or (2) QDs eluted from the MBs.

First, we describe direct detection and quantification of the MB-Ab-S100B-Ab-QD complex without separating the QDs from the MBs. Quantum dots have tunable, size-dependent emission, a broad excitation spectrum, high quantum yield, and do not photobleach.[33] The high quantum yield and resistance to photobleaching allow quantitative analysis over a wide range of exposure times, providing a significant advantage over organic fluorophores. Using this MB-QD sandwich assay (Figure 4A), we are able to detect an S100B concentration of 10 pg mL^{-1} in serum (Figure 4C). Figure 4C shows that the fluorescence signal increases as the S100B concentration increases from 0.1 to 3 ng mL^{-1}; but becomes saturated when the concentration is in the range above 10 ng mL^{-1} close to the designed limits of capture and detection. In

the low concentration range (0.01 to 0.1 ng mL^{-1}), the signal is similar to the background level due to non-specific binding of QDs and autofluorescence of the MBs.

Immunoblot assays are often used for protein analysis. Our immunoblot results (Figure 3 B,C) show detection of free S100B down to a concentration of 10 ng in 10 µL of buffer (1 µg mL^{-1}). The detection limit of our sandwich MB-QD assay is 10 pg mL^{-1} (Figure 4C). Comparison of the detection limits suggests efficient capture by the MBs and fluorescence detection using QDs improve the detection limit of the immunoassay by 100-fold.

The most widely used immunoassay for protein detection is ELISA. Some of the limitations of using ELISA include narrow linear range, assay duration, complexity, limited sample volume, and short enzyme/substrate reaction. We have performed a series of experiments where we varied capture and detection methods to overcome these limitations. For capture, we compared a planar format (a commercial ELISA plate), and bio-functionalized magnetic beads. Then we compared different detection methods, including chemiluminescence detection of enzymes and fluorescence detection of organic fluorophores. Combinations of these methods did not meet the requirements of the assay, such as specificity, sensitivity, and reproducibility (Table 1).

Next, we assessed the assay response following elution of the QDs from the MBs. Scattering from the magnetic beads increases the background signal at shorter wavelengths and autofluorescence of the beads increases the background signal, especially for low concentration detection, and restricts the detection limit of the assay. Elution is a commonly used method for protein purification and immunoprecipitation, and enhanced detection resolution of the MB-QD sandwich assay has been reported following elution using imidazole and urea (see Supplemental information, Table S1).[12, 24-32]

For the MB-Ab-S100B-Ab-QD conjugates, the fluorescence signal for detection of 3 ng of protein is 20 % above the background (control) signal (Figure 4C). Releasing the QDs from the immunocomplex can overcome this low signal-to-noise ratio. The challenging issue is that commonly used elution (immunoaffinity separation/protein denaturant) buffers contain high concentrations of salts, surfactants, or organics. Such buffer compositions can cause QDs to aggregate, precipitate, and lose fluorescence due to the complex surface chemistry and disruption of the lipid encapsulation layer.

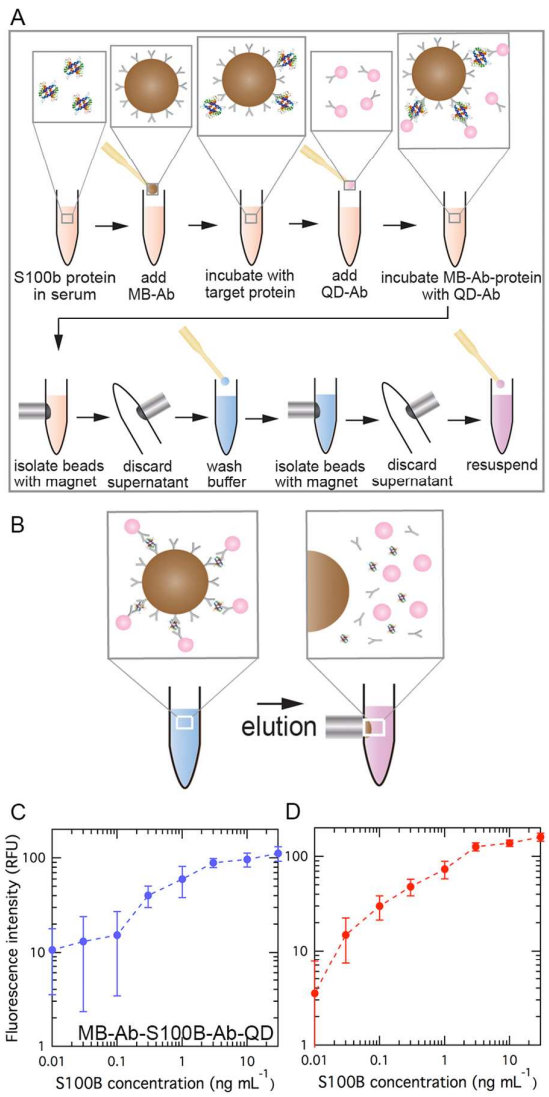

Figure 4. (A) Schematic illustration of the MB-QD sandwich assay for capture and detection of S100B protein. The MB-Ab conjugates are incubated with S100B spiked human serum and subsequently incubated with QD-Ab conjugates for detection. The immunocomplex is re-suspended in buffer for fluorescence measurement after magnetic isolation and washing. (B) Schematic illustration of QD elution. Elution buffer is used to disrupt the non-covalent bonds between the following components: MB-pG, capture Ab, S100B, detection Ab, pG-QD. After magnetic isolation, the supernatant is collected for fluorescence measurements. (C) Fluorescence intensity of the complete immunocomplex (MB-Ab-S100B-Ab-QD) for detection of 0.01 - 30 ng mL^{-1} (0.001 - 3 ng in 100 µL serum) of S100B. The fluorescence intensity is corrected for the background (control). N = 5, and error bars represent standard error. (D) Fluorescence intensity for detection of S100B after elution shows improved signal and a wide linear range. N = 5, and error bars represent standard error.

To achieve a good sensitivity, it is critical to select an elution buffer with high QD releasing efficiency along with a capability to stabilize the fluorescence signal of the released QDs. In immunohistochemistry, urea in concentrations up to 10 M is often used to make fixed tissue transparent to visible light while preserving fluorescent signal from labeled cells.[42] Urea disrupts the non-covalent bonds in proteins and serves as a

	Format		Comments
	Capture	Detection	
1	Antibody plated wells	biotinylated-antibody and streptavidin-HRP conjugate	S100B ELISA kit from Millipore EMD; the detection limit for S100B in serum was 2 ng/mL; total assay time: 4.5 hours
2	Magnetic beads	biotinylated-antibody and Streptavidin-HRP conjugate	Using MBs as a platform for capture lowers the detection limit by 6-fold (~300 pg/mL) and facilitates washing; total assay time: 3 hours
3	Magnetic beads	polyclonal antibody and secondary antibody-HRP conjugate	Poor specificity, low sensitivity and poor reproducibility due to high non-specific binding and cross-reactivity of polyclonal antibodies; total assay time: 3 hours
4	Magnetic beads	Fluorescent organic dye-antibody	Using a fluorescently-labelled antibody reduces the assay time; conjugation yields multiple dyes binding to a single antibody and produces unreliable signal; sensitivity and reproducibility is very low; total assay time: 1 hour

Table 1. Comparison of capture and detection formats tested in developing an assay for S100B detection.

protein denaturant. 8M of urea in borate buffer (pH 8.5) successfully released the QDs from MBs complex without degrading QD emission or stability. Elution separates the following components: MB-pG, capture Ab, S100B, detection Ab, pG-QD (Figure 4B). The fluorescence intensity for detection of S100B after elution shows improved signal and wide linear range, from 0.01 to 3 ng mL^{-1} (Figure 4D). Releasing of QDs improved the signal-to-noise ratio of the assay by a factor of 5.

For our MB-QD sandwich assay, MBs and QDs in suspension enabled relatively fast capture and detection of analytes. Solutions of S100B in serum were incubated with MBs for 15 minutes at 37˚C for capture and then incubated for 15 minutes with QDs for detection. In order to minimize the assay time, we eliminated the washing steps between capture and detection. The sandwich complexes were washed three times and elution buffer was added for 20 minutes. The overall assay time was about one hour, which is about 4 times faster than the processing time of a standard ELISA kit.

Conclusions

Here we have demonstrated an assay for capture and detection of S100B protein using magnetic beads and quantum dots. (1) Sandwich binding of a matched pair of antibodies provides highly specific detection. (2) Use of magnetic beads enables quick and easy mixing/washing steps and minimizes the overall assay time to 1 hour. (3) Efficient conjugation of protein G and antibodies to the QDs ensures antibody stability and functionality without additional chemical reagents and purification steps. (4) The assay has a detection limit of 10 pg mL^{-1} and a 3 order of magnitude of the detection range up to 10 ng mL^{-1}. (5) Releasing the QDs from the MB complex avoids limitations associated with bead autofluorescence and light scattering by the beads, and improves sensitivity of the assay. This MB-QD sandwich assay provides a relatively fast and sensitive assay for detection of S100B for mild traumatic brain injury. In comparison to other reports of MB-QD assays,[24, 27, 28, 31] we show high sensitivity and fast assay time for the detection of a target protein in human serum (see Supplemental information, Table S1).

ASSOCIATED CONTENT

Supporting Information

Table S1. Comparison of magnetic bead-quantum dot sandwich assays.

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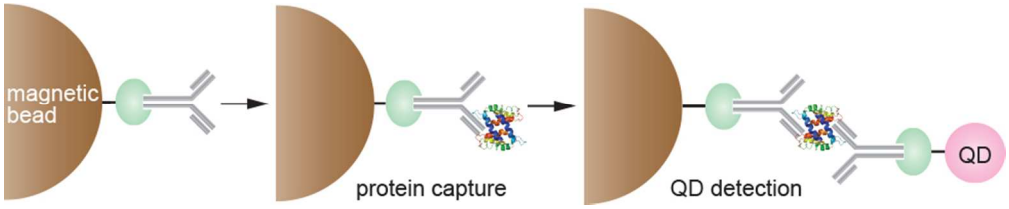

Magnetic bead-quantum dot assay for detection of S100B
82x16mm (300 x 300 DPI)