Self-Assembly Strategy for Reducing Non-Specific Adsorption on Substrates and Application for Quantitative Immunoassay

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Research Article

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Abstract

Background

The development of functionalized surfaces with low non-specific adsorption is important for biomedical applications. To inhibit non-specific adsorption on a substrate, we prepared a novel optical biochip based on a quantum dot fluorescence immunosorbent assay (QD-FLISA), specifically by modifying a layer of dense negatively charged film ($\text{SO}_3^{2-}$) on the glass substrate surface via self-assembly.

Results

Using optimized conditions, we constructed a biochip on functionalized glass substrates to achieve quantitative detection of C-reactive protein (CRP). We subsequently achieved quantitative determination of CRP in the range of 1-1,000 ng/mL, with a limit of detection (LOD) of 1.26 ng/mL or 5.17 ng/mL, using poly(styrene sulfonic acid) sodium salt (PSS) or meso-tetra (4-sulfonatophenyl) porphine dihydrochloride (TSPP) on individually modified glass substrate biochips. The experimental protocol was further optimized and the LOD achieved a sensitivity of 0.69 ng/mL using functionalized TSPP and PSS co-treated glass substrate surfaces for the quantitative detection of CRP.

Conclusions

This work demonstrated an effective and convenient strategy to obtain biochips with low non-specific adsorption properties on functionalized surfaces, thus providing a new approach for creating ultra-high sensitivity microchannels or microarrays on glass substrates.

Background

Non-specific adsorption is a persistent issue that affects the performance of biosensors, because it can decrease detection specificity, reproducibility, and sensitivity [1, 2]. For example, non-specific adsorption of proteins on the surface of a substrate can greatly affect detection by diagnostic instruments. This can cause errors in the detection results, resulting in false positives, which can also affect detection sensitivity [3]. Therefore, effectively inhibiting the non-specific adsorption of biomolecules or bacteria on a substrate surface is a significant challenge in the biomedical industry [4–6]. Antifouling can improve in vitro diagnostic (IVD) analysis, because it reduces non-specific adsorption, providing a better signal-to-noise ratio [7–9]. Recently, some substrate coatings have been developed to inhibit non-specific adsorption, specifically to suppress non-specific adsorption and achieve high-sensitivity detection, including self-assembly of oligomeric polyethylene glycol (PEG) on gold substrates. However, this method cannot eliminate the adsorption of unwanted proteins. In addition, gold and silver typically require complicated reactions when used as substrates [10, 11]. Some substrates have used hydrophilic or hydrophobic treatments to reduce non-specific adsorption on the surfaces of bio-detection chips [12–14].
Although these hydrophilic surfaces can reduce non-specific protein adsorption, they cannot prevent the poor adhesion of cells, bacteria, or other microorganisms [15].

A new approach is currently needed to reduce non-specific adsorption on substrates. Glass slides are common substrates that are widely used in many tests because of their low price and simple preparation [16]. Furthermore, some nanomaterials have been applied to biomedical fields recently [17–20]. In particular, semiconductor quantum dots (QDs) have been widely used in biomedicine due to their unique optical properties [21–23], including high photoluminescence (PL), high quantum yields (QYs), high resistance to photobleaching, and excellent biocompatibility. When biochips are used with glass substrates, some functional groups on the surface will adsorb the QDs materials, because the glass surface contains Si-OH groups [24]; however, but its coverage is limited, causing adsorption of the aqueous QDs or QD-antibody probes. As a result, improving detection sensitivity is difficult.

Biochips based on layer assembly have been studied recently [25–30]; therefore, here we also used glass slide substrates to prepare a dense negatively charged film on the glass surface (functionalized surface), using self-assembly [31]. By successfully reducing the adsorption of the aqueous QDs or QD-antibody probes from the substrate, we prepared a glass substrate-based biosensor with low nonspecific adsorption and achieved high target detection sensitivity. In this work, we designed and prepared a QD-based functionalized surface, as a proof of concept, using a low non-specific adsorption glass substrate biochip with high-sensitivity detection. This glass substrate biochip was fabricated using self-assembly to create poly(styrene sulfonylic acid) sodium salt (PSS) or meso-tetra (4-sulfonatophenyl) porphine dihydrochloride (sulfonated porphyrin or TSPP) substrates, and non-specific adsorption was suppressed by the negatively charged films. High-quality aqueous QDs and QD-antibody probe were then prepared and successfully applied to quantitatively detect the C-reactive protein (CRP) via a quantum dot fluorescence immunosorbent assay (QD-FLISA). We subsequently achieved quantification of the CRP in a range of 1-1,000 ng/mL with limit of detection (LOD) values of 1.26 and 5.17 ng/mL, using individually modified PSS and TSPP glass substrate biochips, respectively. By optimizing the experimental protocol, the CRP was quantified in the range of 1-1,000 ng/mL, using TSPP and PSS to co-treat the glass substrate biochips, and detection sensitivity significantly improved to 0.69 ng/mL, making it nearly 2-fold more sensitive compared to the PSS-modified glass substrate biochip and 7.5-fold more sensitive compared to the TSPP-modified glass substrate biochip. These significant achievements were attributed to the functionalized surface, which could significantly inhibit non-specific adsorption, and provided an excellent platform for ultra-high-sensitive bio-detection in IVD.

Material And Methods

Materials and instruments

Details for the reagents and instruments and the other preparation processes are shown in the supplementary information section.
Results And Discussion

Surface modification of the glass substrate

The glass slide, which is a common substrate, is widely used in various assays owing to its low price and simple preparation. Untreated glass substrates (only washed in piranha solution) directly captured the McCRP1 antibodies for detection, as shown in Fig. S1. However, there was obvious non-specific adsorption (PL intensity reached a count of $3 \times 10^4$) at a detection concentration of 0 ng/mL. Theoretically, when detecting CRP-negative samples (CRP antigen of 0 ng/mL), no significant fluorescence should appear on the detection substrate because no antibody-antigen-antibody sandwich structures formed. However, but obvious fluorescence was observed in the fluorescence microscope image of the blank glass substrate, as shown in Fig. S1. There were numerous carboxyl groups (-COOH) on the aqueous QDs, which are usually present in the solution as -COO$^-$ (negatively charged) in the detection reaction solutions, because buffer solutions are generally weakly basic. The zeta potential of the aqueous QDs was -48.5 mV, and after antibody coupling of the CRP antibody, the zeta potential of the prepared QD-antibody probes increased to -34.2 mV, as it still had a large amount of negative carboxyl charges (-COOH or -COO$^-$) on its surface (see Fig. S2 for details). Afterward, the piranha solution was cleaned off the glass substrate surface to check for the presence of Si-OH; however, its coverage was not sufficiently uniform or dense [32], resulting in non-specific adsorption of the aqueous QDs (Fig. 1a). This was attributed to the special functional groups that were present on the surface of the glass substrate, which created electrostatic and non-specific adsorption sites on the carboxyl groups for the aqueous QDs or QD-antibody probes. Therefore, during the assay process, if there was significant non-specific adsorption on the substrate, then it would likely affect the accuracy of the subsequent assay experiments and the sensitivity of the assay. Thus, to further prepare the functionalized surface to inhibit non-specific adsorption on glass substrates, we utilized the following methods.

Optimization of glass substrate conditions using PSS treatment

To confirm the above points, we conducted the following experiments, as shown in Fig. 1a and d. The fluorescence spectra of the aqueous QDs (the excitation wavelength is 613 nm and the emission wavelength is 405 nm) adsorbed by the glass substrates under different treatment conditions were collected, including the blank-glass substrate, the PDDA (i.e. poly(diallyldimethylammoniumchloride))-glass substrate, the PDDA and PSS-glass substrates, and the PSS-glass substrate (see details in the experimental section). As shown in Fig. 1a, the blank-glass substrate (blue line) resulted in significant adsorption of the aqueous QDs with a fluorescence intensity of $1.5 \times 10^4$ (counts). The fluorescence intensity of the PDDA-glass substrate increased as a result of electrostatic adsorption of the positively charged PDDA onto the aqueous QDs, owing to the negative surface charges introduced on the glass substrate. Adsorption of the aqueous QDs on the glass substrate was weaker after the PDDA was overlaid with PSS. The adsorption of the aqueous QDs was significantly reduced after the direct PSS overlay treatment on the glass substrate, especially compared to the blank-glass substrate. Therefore, we
determined that the introduction of certain negatively charged substances on the glass substrates may have reduced the adsorption of the aqueous QDs on the glass substrate surfaces. Next, we used a PSS solution with a negative charge to treat the glass substrates, and promote self-assembly, and we fabricated 1-6 layers of PSS films on the glass substrates. As shown in Fig. 1b, the adsorption of the aqueous QDs on the glass substrates was weaker with an increasing number of PSS layers. With fewer modified PSS layers, the glass substrate surfaces were not completely covered with negative charges, resulting in some adsorption. Thus, non-specific adsorption of the aqueous QDs was significantly inhibited when four layers of PSS were used. The glass substrate surface treated with four PSS layers resulted in a relatively dense negative charge surface on the glass substrate, which could effectively inhibit the adsorption of the aqueous QDs on the glass substrate. Subsequently, the PSS films treated with different numbers of layers (1, 3, and 4 layers) were compared, as shown in Fig. S3, and we found that the coverage density of the PSS films on the substrate increased with an increasing number of PSS layers. Thus, the root-mean-square (RMS) roughness gradually decreased by 3.41, 2.18, and 1.6 nm. However, the overall PSS films showed poor film properties and slight decreases in density.

To further improve the immobilization efficiency and the densification of the PSS film, we modulated PSS film formation by changing the ionic strength of the PSS solution, by adding a certain concentration of inorganic salt ions to the solution [33, 34]. As shown in Fig. S3d, a highly dense PSS film was treated on the surface of the glass substrate by 0.5 mol/L of NaCl solution addition. The non-specific adsorption of the aqueous QDs on the glass substrate was reduced when it was treated with PSS containing 0.5 mol/L of NaCl solution, as shown in Fig. 1c and f. The glass substrate was treated with PSS and NaCl solution, and then annealed at 140ºC to improve the density and stability of the PSS films, allowing for better fixation of the PSS on the glass substrate surface in Fig. S4. We then assessed the fluorescence spectra of the aqueous QDs adsorbed on glass substrates fabricated using different treatment conditions, including a glass substrate treated with four layers of PSS solution, a glass substrate treated with four layers of PSS with NaCl solution, a glass substrate treated with PSS containing NaCl solution and then annealed at high-temperature and a glass substrate treated by spin-coating PSS solution. As shown in Fig. 1c and f, the adsorption of the aqueous QDs on the glass substrate after the addition of NaCl solution and annealing treatment was significantly reduced. We speculated that the addition of inorganic salt ions improved the density of the PSS film, and annealing caused the PSS to become immobilized on the glass substrate. When the polymer electrolyte solution (PSS solution) did not contain inorganic salt ions or the concentration was low, the polyelectrolyte chains had a more extended conformation in the solution because of electrostatic repulsions between the adjacent fragments. This caused them to adsorb onto the glass substrate, forming a thin film layer. In the presence of certain inorganic salt ions, the electrolyte molecules adopted a more curled structure and formed a denser film after adsorption onto the substrate due to shielding effect of the charges on the polyelectrolyte by the counterbalance ions [35]. The density of the PSS film treated by spin-coating was significantly influenced by the solution concentration and spin-coating speed, which will be discussed in a subsequent publication. We determined that the final treatment method was PSS with NaCl solution after high-temperature annealing treatment, and this approach was effective in reducing the adsorption of aqueous QDs on the glass
substrate. Compared to the untreated glass substrate, this method could reduce adsorption by about 300-fold (1.5×10^4 counts vs 51 counts). These results were attributed to the formation of the negatively charged \( \text{SO}_3^{2-} \) film on the glass substrate, which inhibited the adsorption of the aqueous QDs (with carboxyl groups on the surface).

In addition, the above method was verified using SDS (sodium dodecyl sulfate), SLS (sodium lauryl sulfate), and other negatively charged ions (\( \text{SO}_3^{2-}, \text{SO}_4^{2-} \)) using a monolayer of negatively charged film, to evaluate the aqueous QD adsorption experiments. Fig. 2 shows the chemical structures of the four substances, where the surface modification of the glass substrates by PSS, SDS, SLS, and TSPP inhibited the aqueous QDs, indicating that the negatively charged ions could inhibit the adsorption of aqueous QDs overall. In particular, TSPP (sulfonated porphyrin) contained four sulfonic acid groups, which increased the overall negative charge coverage, creating an obvious inhibition effect for the aqueous QDs.

**Optimization of the glass substrates using TSPP treatment**

To verify the effect of TSPP on the non-specific inhibition of the aqueous QDs, we conducted the following experiments. The glass substrates were treated with different TSPP solution layers, and we then evaluated the adsorption effect on the aqueous QDs. As shown in Fig. S5, the adsorption of the aqueous QDs on the glass substrate gradually decreased with an increasing number of TSPP layers. When fewer TSPP layers were modified, the glass substrate surfaces were not completely covered by negative charges, which caused some adsorption. The non-specific adsorption of the aqueous QDs was significantly inhibited when the two TSPP layers were modified. Treatment of the glass substrate surface with two TSPP layers resulted in ideal negative charge modification of the glass substrate surface, which effectively inhibited the adsorption of the aqueous QDs by the glass substrate. Compared to the surface modification of the glass substrate using PSS solution, the TSPP substance increased the coverage of the glass substrate due to the large amount of negative sulfonic acid group charges, which likely reduced adsorption by ~400 times (~38 counts) compared to the untreated glass substrate (1.5×10^4 counts).

**Optimization of glass substrate conditions using TSPP and PSS treatments**

Because of some interactions between the TSPP solution and the aqueous QDs solution [36], and to reduce the effects of TSPP, we initially treated the glass substrate with two layers of TSPP and then four layers of PSS, which created a distance between the TSPP and QDs on the glass substrate. As shown in Fig. S6, we compared the adsorption of the 2-layer TSPP, 4-layer PSS, and 2-layer TSPP and 4-layer PSS treated glass substrates for the adsorption of aqueous QDs. The glass substrates under all three treatment conditions could effectively inhibit the adsorption of aqueous QDs. Compared to the untreated glass substrate, adsorption was reduced by approximately 400 times for the TSPP and PSS-glass substrates created using the self-assembly method.

**AFM characterization of the different glass substrates**
Surface roughness is an important index for evaluating surface morphology, and the morphology and surface roughness of the samples were evaluated using atomic force microscopy (AFM). The surface microstructures of the blank-glass and functionalized glass substrate surfaces were characterized, with a sample area of 5×5 µm. As shown in Fig. 3a, the surface of the blank-glass substrate was be at with an RMS roughness value of 0.31 nm. The charges on the glass substrate were tested using a solid zeta potentiometer and we determined that the zeta potential on the surface of the blank-glass substrate was -100.4 mV. As shown in Fig. 3b, the two-dimensional (2D) AFM image indicated that some granular material was deposited on the PSS-glass substrate surface, indicating that PSS was successfully modified on the glass substrate. The three-dimensional (3D) AFM image indicated that the PSS-modified glass substrate surface was flat and its RMS roughness value was 1.47 nm. The negative charge on the PSS-modified glass substrate increased to -105.5 mV, indicating that the negative charges were successfully modified on the glass substrate.

The 2D AFM image verified the successful modification of the glass substrate by TSPP with a large amount of granular material deposited on the surface of the TSPP-glass substrate, as shown in Fig. 3c, while the 3D image indicated an RMS value of 3.53 nm. The zeta potential of the TSPP-glass substrate was -114.7 mV, which is increased compared to the PSS-glass substrate. As shown in Fig. 3d, the 2D AFM image showed that the TSPP and PSS-glass substrate had some granular material deposited on the surface, and the particle density increased. In addition, the 3D AFM image showed an increase in the surface roughness of the glass substrate after TSPP and PSS co-modification with an RMS roughness value of 4.36 nm. A zeta potential of -122.0 mV was determined for the TSPP and PSS-glass substrate, which increased compared to the glass substrates individually modified with PSS and TSPP.

Optimization of the coating conditions on the glass substrate biochips and detection of the C-reactive protein

We explored the McCRP1 antibody coating on the PSS-glass substrate, and to obtain more accurate results for the coating effect, we utilized QD-antibody probes instead of using coating antibodies. There was no obvious adsorption of the aqueous QDs on the PSS-glass substrate, indicating that the adsorption that did occur when the glass substrate was treated using the QD-antibody probes was because the antibodies (on the QD-antibody probes) were immobilized on the glass substrates as a result of intermolecular forces. Next, we used different methods to coat the QD-antibody probes, at 25°C for 2 h, 37°C for 2 h, and 4°C overnight. The coating results were evaluated, and the results are shown in Fig. 4a, showing that the coating effects at 25°C for 2 h and overnight at 4°C were the same. However, while a stronger fluorescence intensity was found on the PSS-glass substrate at 37°C for 2 h (red line). This indicated that the increase in temperature caused an increase in the adsorption of antibodies on the glass substrate, which effectively improved the coating efficiency. Based on the above results, we optimized the coating reactive time to 37°C and selected 2 h, 4 h, 6 h, 8 h, and 12 h (overnight) for the coating antibody tests. The results are shown in Fig. 4b, indicating the that adsorption of the antibodies on the glass substrates was noticeable when the substrates were coated overnight at 37°C, and the coating effect
improved. The adsorption of aqueous QDs was evaluated under this condition and we found no obvious non-specific adsorption on the glass-glass substrate at 37°C. This indicated that the fluorescence was due to the intermolecular forces between the antibodies on the QD-antibody probes and the glass substrate surface, causing the antibodies to become immobilized on the glass substrates. As shown in Fig. S7, the McCRP1 antibody is coated on the PSS-treated glass substrate, while the SO₃-rich surface has an improved ability to bind the captured antibodies. The McCRP1 antibody coating was explored using a TSPP-glass substrate, based on the results of the above study. Thus, the QD-antibody probes were coated overnight at 37°C, and as shown in Fig. S9a, the TSPP-glass substrate adsorbed readily to the QD-antibody probes with a good immobilization effect. The evaluation of the adsorption experiments using this substrate on the aqueous QDs under this condition revealed that this high-temperature condition did not cause significant non-specific adsorption on the glass substrate.

**Fluorescence microscope images of different glass substrate biochips**

Fluorescence microscope images of the glass substrate biochips after different treatments were obtained, and the results are shown in Fig. 5. The surface-modified glass substrate biochips showed no significant adsorption for the aqueous QDs, and no significant fluorescence was observed, as shown in Fig. 5a-c. The three glass substrate biochips were used to detect concentrations of 0 and 1,000 ng/mL, and fluorescence microscopy images were obtained. No obvious fluorescence was observed on the glass substrate biochips for the negative sample (0 ng/mL); however, while fluorescence intensity was observed at the concentrations of 1,000 ng/mL. As shown in Fig. 5d-f, the PL intensities of the different glass substrate biochips were nearly identical to the background noise when the CRP antigen concentration was 0 ng/mL. This indicated that these glass substrate biochips had almost absolute zero background for detection [37]. Compared to the TSPP-glass substrate biochips, the PL intensities of the PSS-glass substrate biochips and the TSPP- and PSS-glass substrate biochips were significant for the high-concentration assay.

**Quantitative detection of CRP using PSS-glass, TSPP-glass, and TSPP and PSS-glass substrate biochips**

Based on the above optimized experimental conditions, PSS-glass, and TSPP-glass, and TSPP and PSS-glass substrate biochips were prepared to detect different concentrations of CRP standard antigens within 1-1,000 ng/mL. The schemes of the different glass substrate biochips are shown in Fig. 6a, d, and g, after the CRP assay. Six points at different locations on the glass substrate biochips were selected (see Scheme 1 for details) and detected using the custom-built fluorescence detection system. Generally, PL intensity gradually increases with increasing CRP antigen concentration, as shown in Fig. 6. However, after averaging the fluorescence intensity at the peak location, the CRP antigen concentration (X) and the corresponding fluorescence intensity (Y) showed linear regressions. As shown in Fig. 6b and c, the linear range for CRP antigen detection using the PSS-glass substrate biochips as 1-1,000 ng/mL with a standard curve of \( \lg Y = 3.27 + 0.37 \lg X \) and a linear correlation \( (R^2) \) of 0.988. According to the
calculations (see details in the experimental section), the LOD was 1.26 ng/mL. For the TSPP-glass substrate biochips, the numerical data from Fig. 6e and f best fit with the following calibration curve, \( \log Y = 3.04 + 0.33 \log X \) with \( R^2 = 0.984 \) (n = 3). Owing to the effects of the TSPP solution on the fluorescence intensity of the QDs, the overall fluorescence intensity for CRP detection was lower with a poor fluorescence peak shape (TSPP solution had a small fluorescence peak at 663 nm) [38], making it difficult to improve the sensitivity, which was only 5.17 ng/mL. As shown in Fig. 6h and i, the linear range for CRP antigen detection using the TSPP- and PSS-glass substrate biochips was 1-1,000 ng/mL, and the standard curve was \( \log Y = 3.8 + 0.21 \log X \) with a linear correlation of 0.992. As shown in Fig. 6h, the difference in fluorescence intensity was more noticeable at low concentrations, and the LOD was calculated to be 0.69 ng/mL. As shown in Table 1, the sensitivity increased nearly 2-fold compared to the PSS-glass substrate biochips and nearly 7.5-fold compared to the TSPP-glass substrate biochips. Thus, the sensitivity reported in this study was significantly improved compared to common types of biosensors and biochips [39–41].

<table>
<thead>
<tr>
<th>Different biochips</th>
<th>Material</th>
<th>Range</th>
<th>LOD (ng/mL)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>enzyme-linked immunosorbent assay</td>
<td>HRP</td>
<td>2.2-100 ng/mL</td>
<td>2.2</td>
<td>39</td>
</tr>
<tr>
<td>polymethylmetacrylate (PMMA) chip</td>
<td>fluorochrome (DY647)</td>
<td>0.1-50 µg/L</td>
<td>4</td>
<td>40</td>
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<tr>
<td>giant magnetoresistance (GMR) sensor</td>
<td>magnetic nanoparticles</td>
<td>3 ng-350 µg/L</td>
<td>1</td>
<td>41</td>
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<tr>
<td>PSS-glass substrate biochip</td>
<td>CdSe/ZnS QDs</td>
<td>1-1,000 ng/mL</td>
<td>1.26</td>
<td>This paper</td>
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<tr>
<td>TSPP-glass substrate biochip</td>
<td>CdSe/ZnS QDs</td>
<td>1-1,000 ng/mL</td>
<td>5.17</td>
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<td>PSS and TSPP-glass substrate biochip</td>
<td>CdSe/ZnS QDs</td>
<td>1-1,000 ng/mL</td>
<td>0.69</td>
<td>This paper</td>
</tr>
</tbody>
</table>

### Suitability evaluation of different biochips

Subsequently, we evaluated the specificity properties of the different glass substrate biochips. A series of common interference factors in blood, such as L-cysteine, DL-homocysteine, glutathione, and alkaline phosphatase (ALP), as well as inflammatory factors (procalcitonin (PCT) and serum amyloid A (SAA)) were configured at a concentration of 10 µg/mL, and 100 ng/mL for the CRP antigens. These were subsequently tested in the following immunoassay using the different glass substrate biochips. As shown in Fig. 7 (series 1, 2, and 3), compared to the PL intensity of the CRP system, we detected negligible effects on PL intensity due to interference factors. Thus, the proposed glass substrate biochips
possessed good selectivity without apparent interference from the nonspecific adsorption of other antigens.

Recovery experiments for CRP antigens were conducted to evaluate the accuracy of the three glass substrate biochips, through the addition of different concentrations of CRP antigens in human negative serum. High, medium, and low concentrations (500, 50, 5 ng/mL) of CRP antigens were selected for the assay. As shown in Table 2, the recovery rates of the CRP samples on the PSS-glass, TSPP-glass, and TSPP and PSS-glass substrate biochips ranged from 103.1 to 107.2%, 88.4 to 110.8%, and 99.5 to 104.7%, respectively, resulting in a coefficient of variance (CV) of less than 15%. Hence, these results indicated that these biochips were reliable for detecting CRP antigens.

Table 2
Comparison of different biochips for CRP detection (N = 6).

<table>
<thead>
<tr>
<th>Different biochips</th>
<th>Spiked (ng/mL)</th>
<th>Real value (ng/mL)</th>
<th>Recovery (%)</th>
<th>CV (%)</th>
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<tbody>
<tr>
<td>PSS-glass substrate biochip</td>
<td>5</td>
<td>5.15</td>
<td>103.1</td>
<td>7.3</td>
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<tr>
<td></td>
<td>50</td>
<td>53.69</td>
<td>107.4</td>
<td>8.11</td>
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<td></td>
<td>500</td>
<td>536.21</td>
<td>107.2</td>
<td>12.36</td>
</tr>
<tr>
<td>TSPP-glass substrate biochip</td>
<td>5</td>
<td>5.54</td>
<td>110.8</td>
<td>9.98</td>
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<tr>
<td></td>
<td>50</td>
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<td></td>
<td>500</td>
<td>495.08</td>
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</table>

Conclusion

Reducing non-specific adsorption is one of the important means of improving assay sensitivity of the assay, and this approach is needed for the future development of IVD analysis [42]. In this study, we used common glass slides as substrates, utilizing self-assembly to treat the functionalized glass substrate surfaces, thus modifying the glass surfaces with a dense layer of negatively charged film (SO$_3^{2-}$). This ultimately reduced the adsorption of the substrate for the probe material. A glass substrate biochip with low non-specific adsorption was prepared, achieving highly sensitive quantitative detection of CRP based on QD-FLISA. The quantitative detection range of CRP on the glass substrate chip using TSPP and PSS co-treatment was 1-1,000 ng/mL, and the LOD reached 0.69 ng/mL. Therefore, this approach provided an
effective solution for developing functionalized surfaces with low non-specific adsorption properties, which may be used for high-sensitivity detection in biomedical applications.

**Abbreviations**

2D: two-dimensional; 3D: three-dimensional; Ab: antibody; AFM: atomic force microscopy; Ag: antigen; CRP: C-reactive protein; ELISA: enzyme-linked immunosorbent assay; IVD: in vitro diagnostic; LOD: the limit of detection; PDDA: poly(diallyldimethylammonium chloride); PEG: polyethylene glycol; PL: Photoluminescence; PSS: poly(styrene sulfonic acid) sodium salt; QDs: quantum dots; QD-FLISA: quantum dot fluorescence-linked immunosorbent assay; QYs: quantum yields; R: the correlation coefficient value; RMS: root-mean-square; SD: standard deviation; SDS: sodium dodecyl sulfate; SLS: sodium dodecyl sulfate; sulfo-NHS: N-Hydroxysulfosuccinimide; TSPP: *meso*-Tetra (4-sulfonatophenyl) porphine dihydrochloride.

**Declarations**

**Consent for publication** Not applicable

**Availability of data and material** Not applicable

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ contributions**

Lv participated in the design of the study, optimized the reaction conditions of biochip for detecting CRP, performed the statistical analysis and drafted the finalized the manuscript. Wu and Guo explained the experimental phenomena and modified the manuscript. Zhao and Fan tested the performance of the biochip. Li J and Xu made hydrophobic CdSe/ZnS QDs to surface modification. Mao prepared the QDs-antibody detecting probes. Li N and Shen provided the hydrophobic CdSe/ZnS QDs. Li L initiated the study, supervised the experiments and data interpretations. All authors read and approved the final manuscript.

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References


Supplementary

Schema is available in supplementary section

Figures
Figure 1

Fluorescence spectra (a) and corresponding fluorescence intensity (d) of different reagent treatments of glass substrates for aqueous QDs adsorption. Fluorescence spectra (b) and corresponding fluorescence intensity (e) of different PSS layers of treated glass substrates for aqueous QDs adsorption. Fluorescence spectra (c) and corresponding fluorescence intensity (f) of different processing methods of glass substrates for aqueous QDs adsorption.
Figure 2

Fluorescence intensity of different reagent treatment of glass substrates for aqueous QDs adsorption, including PSS, SDS, SLS, and TSPP. The corresponding chemical structures of PSS, SDS, SLS, and TSPP.
Figure 3

Atomic force microscopy (AFM) image of glass substrates with different processing methods, including blank-glass substrate (a), PSS-glass substrate (b), TSPP-glass substrate (c), and TSPP and PSS-glass substrate (d).
Figure 4

Optimization to capture McCRP1 antibody for PSS-glass substrate biochips (a) and (b), and corresponding fluorescence intensity (c) and (d).

Figure 5

Fluorescence microscope images of PSS-glass substrate biochips (a), TSPP-glass substrate biochips (b), and PSS and TSPP-glass substrate biochips (c), and the corresponding PL intensity of PSS-glass substrate biochips (d), TSPP-glass substrate biochips (e), and PSS and TSPP-glass substrate biochips (f).
Figure 6

The glass substrate biochips schemes by PSS treatment (a), TSPP treatment (d), and TSPP and PSS co-treatment (g). The photoluminescence spectra for determination of CRP antigens by PSS-glass substrate biochip (b), TSPP-glass substrate biochip (e), and PSS and TSPP-glass substrate biochip (h). The corresponding calibration curves for CRP quantitative detection of 0-1,000 ng/mL (c), (f) and (i). Error bars indicate the standard deviations of three independent experiments.
Figure 7

Cross-reaction and specificity of PSS-glass substrate biochips (Series 1), TSPP-glass substrate biochips (Series 2), PSS and TSPP-glass substrate biochips (Series 3).

Supplementary Files

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