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Biocompatible polymers for antibody support on gold surfaces

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Abstract

The elimination or minimization of non-specific protein adsorption from serum is critical for the use of surface plasmon resonance (SPR) sensors for in vitro and in vivo analysis of complex biological solutions. The ultimate goals in this application are to minimize non-specific adsorption of protein and to maximize analyte signal. A reduction of the non-specific protein adsorption from serum of up to 73% compared to carboxymethylated-dextran 500 kDa (CM-dextran) was achieved following a survey of eight biocompatible polymers and 10 molecular weights of CM-dextran. These coatings minimize non-specific adsorption on the sensor while also serving as immobilization matrices for antibody fixation to the probes. Polymers including polysaccharides: CM-dextrans, CM-hyaluronic acid, hyaluronic acid, and alginic acid were investigated. Humic acid, polylactic acid, polyacrylic acid, orthopyridyldisuldfide–polyethyleneglycol–*N*-hydroxysuccinimide (OPSS–PEG–NHS), and a synthesized polymer; polymethacrylic-acid-*co*-vinyl-acetate (PMAVA) were also used. The non-specific protein adsorption reduction was measured over a 14 day period at 0 °C for each polymer. Calibration curves using some of these polymers were constructed to show the performance and low detection limit possibilities of these new antibody supports. For many of the polymers, this is the first demonstration of employment as an antibody support for an optical or surface active sensor. CM-dextran is the polymer offering the largest signal for the antigen detection. However, the biocompatible polymers demonstrate a greater stability to non-specific binding in serum. These biocompatible polymers offer different alternatives for CM-dextran.

Keywords: Biopolymer; Surface plasmon Resonance; Non-specific binding; Protein fouling

1. Introduction

The future of surface plasmon resonance (SPR) biosensor as a medical tool to monitor protein and biological markers relies on the sensor's stability in a highly complex media. For example, the stability of the sensor in whole blood is required to obtain a quick diagnosis of myocardial infarctions. One of the major benefits of SPR biosensors is that any disease or events that releases markers in any biological fluids could be monitored using the same SPR sensor template, but using different antibodies on the surface. To attach antibodies on the surface, a linker between the gold surface and the antibodies must be employed. However, that linker is also highly prone to non-specific binding (NSB) from serum proteins. CM-dextran is commonly used, which is a polymeric support with a high density of carboxylic acids. However, CM-dextran does not reduce the non-specific binding to a level low enough to detect an antigen in full serum (serum protein concentration of 84 mg/mL). Among the thousands of polymers available, some will offer better stability than CM-dextran to NSB and some offer a sensitivity to the biological markers that will be as good or better than CM-dextran. Thus, the goal is to find biocompatible polymers that offer a net improvement in stability and sensitivity relative to CM-dextran. In this study, some polymers that were already proven as biocompatible on medical instruments are investigated to test the potential of these biocompatible polymers to serve as an antibody support for in vitro and in vivo sensors.

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SPR sensors are sensitive to changes in refractive index near the gold surface supporting the surface plasmon wave, independent of the cause of the refractive index change. The immobilization of antibodies on the surface allows SPR sensors to be used to track the specific binding of antigens of interest. However, NSB of molecules to the surface will cause a signal that will mask the signal from analytes of interest. Many useful applications using SPR sensors are inhibited by this non-specific signal; for example, analyte measurement in whole blood, blood plasma, or serum has not yet been achieved due to the NSB of blood-borne proteins on the sensor surface. The non-specific signal caused by protein adsorption has been studied before [1–5]. Currently, the only SPR-based measurements in blood samples are the coagulation of both whole blood [6–9] and blood plasma [10]. Analytical measurements have been made in synovial fluids [11], diluted or pre-treated urine [12,13], pre-treated blood for analyte extraction [14], and diluted serum with high analyte concentrations [15–19]. This article presents efforts to reduce serum NSB of SPR sensors using sensor coatings that simultaneously provide binding sites for immobilized antibodies and reduce NSB.

SPR sensors offer the possibility of real-time monitoring of protein adsorption to surface coatings [5,20]. Properties of polymer coatings for NSB on SPR sensors have been reported [2,21–27]. Self-assembled dextran monolayers are protein-resistant compared to uncoated surfaces although dextran does not sufficiently reduce NSB allow the detection of very low analyte concentrations in very complex matrices, such as blood [28]. Concurrently, Mrksich et al. found that self assembled monolayers (SAM) comprising of hexa(ethyleneglycol) and methyl groups can resist protein adsorption [29]. A thorough evaluation of surface coating properties to reduce NSB has not been reported.

The preparation of SPR sensors with different biocompatible polymers to eliminate NSB was previously reported [30,31]. The biocompatible polymers studied have carboxylic acids on their backbone that allow antibody attachment. The polymers were proven to attach sufficient levels of antibodies to allow detection of biologically-relevant concentrations of antigens. Humic acid [32], hyaluronic acid [33,34], carboxymethylated hyaluronic acid (CM-hyaluronic acid), alginic acid [35], polyacrylic acid [36], orthopyridyldisuldfide–polyethyleneglycol–*N*-hydroxysuccinimide (OPSS–PEG–NHS) [37], and polymethacrylic-acid-*co*-vinyl-acetate (PMAVA) were previously reported as being effective at reducing NSB. Although these polymers were not investigated as supports for antibody attachment for biosensor applications. Biocompatible polymers do not cause damage or adversely affect biological function. Therefore in the SPR sensor case, blood coagulation is prevented on the sensor by the biocompatible polymer. The first step of blood coagulation is the adsorption of blood-borne proteins to the surface. Therefore, desirable polymers necessarily reduce protein adsorption. DL-Polylactic acid (PLA) [38] is a biocompatible polymer without carboxylic acids on the backbone. It is used as a reference here because antibodies do not bind to the PLA when it is used to coat the probe. All polysaccharides studied reduced NSB compared to carboxymethylated-dextran 500 kDa (CM-dextran) [31].

This work investigates 18 different layers for antibody attachment for reduction of NSB caused by bovine serum onto a SPR fiber-optic sensor. The coatings were proven to attach enough antibodies to allow detection of biologically relevant concentrations of myoglobin (MG). The effects of the pH and temperature of the antibody solution during reaction with the linker on the subsequent NSB and sensing performance of the probes was studied. The calibration of SPR probes coated with some of the polymers is also demonstrated. The goal here is to simultaneously minimize NSB and maximize analyte signal.

2. Experimental

2.1. Sensor preparation

The manufacture of the fiber-optic SPR sensors and the SPR system used in this study has been described previously [30,39,40]. Fiber optic sensors are employed because of their adaptability to in vitro and in vivo measurements compared to prism based geometries for SPR sensors.

2.2. Layer preparation

The preparation and synthesis of CM-dextran, CM-hyaluronic acid, hyaluronic acid, alginic acid, humic acid, PLA, polyacrylic acid, PMAVA, and OPSS–PEG–NHS polymer layers have been extensively described by Masson et al. [30].

2.3. Antibody reaction

Probes with the polymers described previously are reacted to attach antibodies to the two analytes of interest, myoglobin (MG) and cardiac Troponin I (cTnI), on the sensor's surface. OPSS–PEG–NHS is reacted as previously described [37]. For MG detection, the surface is activated by immersion in 1:1 aqueous solutions of 0.4 mol L^{-1} *N*-ethyl-*N*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and 0.01 mol L^{-1} *N*-hydroxysuccinimide (NHS) for 10 min. An amine coupling is performed on this activated surface by reaction with a 700 μg/mL solution of human anti-myoglobin (ICN Biomedicals, polyclonal rabbit antiserum to human MG; K_A and k_A are not available). The surface is reacted in a pH 4 (10 mmol L^{-1} sodium acetate buffer) and 37 °C antibody solution for 20 min. For cTnI detection, the surface is reacted with a 100 μg/mL human anti-cardiac Troponin I (Spectral Diagnostics) solution at pH 6 (10 mmol L^{-1} sodium acetate buffer) and 37 °C for 20 min. These conditions were found to be optimal in previous studies [39]. The

non-specifically bound antibodies are washed away and the non-reacted sites on the polymers are deactivated by rinsing the probe with an aqueous solution of 1 M ethanolamine at pH 8.5, for 10 min.

2.4. MG and cTnI measurements

MG (ICN Biomedicals) and cTnI (Spectral Diagnostics) solutions were prepared in HEPES buffered saline (HBS) at pH 7.4. MG is received as a lyophilized powder and is reconstituted to 1 mg/mL using 18 MΩ deionized water. MG is stored at –20 °C for extended periods of time. A stock solution at 500 ng/mL is prepared followed by dilutions in HBS pH 7.4 to the desired concentration. HBS is composed of 150 mmol L^{-1} NaCl, 10 mmol L^{-1} HEPES, 3.4 mmol L^{-1} EDTA, and 0.005% Tween 20 (surfactant) in 18 MΩ deionized water. The pH of the HBS solution was adjusted to 7.4 using 2 M NaOH. cTnI is received at 1.22 mg/mL in 20 mmol L^{-1} Tris–HCl, 500 mmol L^{-1} NaCl, 10 mmol L^{-1} β-mercaptoethanol at pH 7.5. This solution is stored at −20 °C for extended periods of time. A stock solution is prepared from the previous solution at 488 ng/mL in HBS pH 7.4. The stock solutions for MG and cTnI are stored at 4 °C until use and are prepared daily. cTnI solution is diluted to the desired concentration with HBS at pH 7.4. MG and cTnI solutions are thermally equilibrated in a water bath at 25 °C for 30 min before analysis. The sensor is equilibrated for 15 min in HBS before use. The SPR signal is monitored for 5 min in a static HBS, pH 7.4 solution for 5 min, and then transferred to the analyte solution. The analyte measurement is performed in a static solution. The sensor is exposed to HBS after analyte measurement for regeneration. Up to five consecutive measurements can be obtained for each sensor before antibody degradation reduces the probe sensitivity. The data acquisition is performed at a rate of 1 data point every 3 s. Each data point is an accumulation of three measurements.

2.5. Non-specific binding monitoring

The sensors with the different coatings are prepared as previously described [30]. Anti-MG functionalized sensors are placed in a bovine serum (ICN Biomedicals) solution at 0 °C. Serum comes from adult animals and was sterile filtered through a 0.2-μm membrane filter. The serum protein concentration is 84 mg/mL. NSB in a static serum solution is measured. A daily measurement of λ_{SPR} is made for 14 days while the sensor in immersed in the serum solution. NSB is measured in real-time, therefore no rinsing of the sensor surface is performed. The daily measurement is an average of 50 data points taken every 250 ms. Including the time required to install the sensor on the system and save the acquisition, the measurement takes approximately 30 s. Measuring every sensor in the set takes approximately 10 min, therefore the measurements are considered to be simultaneous relative to the timescale of the experiment. The serum in these experiments comes from a single batch; thus, the commonly employed CM-dextran support serves as an implicit standard against which all measurements can be compared. The amount of NSB is reported as a shift of λ_{SPR} during the NSB process and as a percentage compared to the degree of NSB observed in our previous study using CM-dextran 500 kDa [31]. The shift is calculated by subtracting λ_{SPR} at time 0 to λ_{SPR} at the later time. The shift is the average signal for the final 5 days of the experiment. Doing the NSB experiment at 0 °C was found to reduce the experiment-to-experiment variations and make more meaningful comparisons among the sensor coatings [31].

3. Results

3.1. NSB experiment

In order to use the SPR sensors in complex solutions such as serum or whole blood, the signal from the serum or blood must be minimized so that the signal due to dissolved analytes of interest can be obtained cleanly. CM-dextran sensors will foul quickly in complex solutions. For example, such sensors were placed in a bovine serum solution (84 mg/mL proteins) for 10 min [30], and the signal resulting from NSB from the bovine serum was around 10–100 times the signal resulting from specific binding of cTnI to immobilized an-

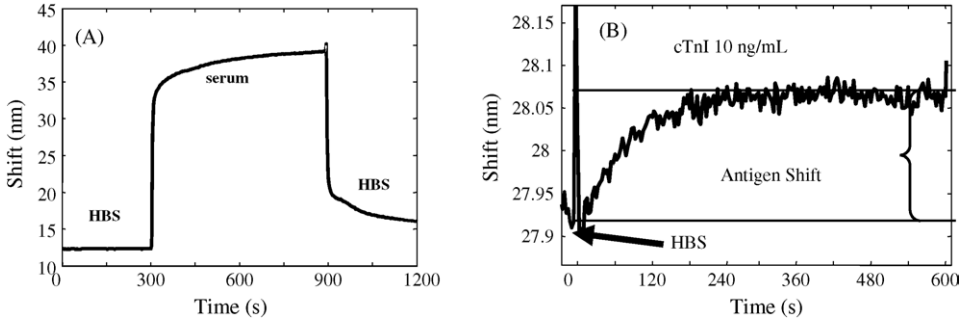

Fig. 1. The signal when the sensor is placed in contact with bovine serum at 84 mg/mL and 25 °C (A) is much larger than the signal for cTnI detection at 10 ng/mL (B). The signal from protein adsorption in serum did not reach equilibrium after 10 min exposure. The signal for cTnI binding reached equilibrium after less than 5 min. The signal from NSB in serum is also partially irreversible as seen when the sensor was washed in HBS after exposure to bovine serum.

Table 1
CM-dextran layer performance for cTnI detection and NSB in bovine serum 84 mg/mL

CM-dextran MW (kDa)	25 ng/mL cTnI shift (nm)	NSB Shift (nm)	%NSB compared to CM-dextran 500 kDa	NSB surface coverage (ng/cm^2)	Performance factor ($\times 10^{-3}$)
3	N/A	7 ± 2	27 ± 9	231	N/A
5	N/A	11 ± 2	42 ± 11	352	N/A
17.5	N/A	12 ± 2	46 ± 11	391	N/A
25	0.000 ± 0.008	12 ± 2	45 ± 11	379	0
75	0.000 ± 0.008	14 ± 6	53 ± 24	448	0
150	0.026 ± 0.008	15 ± 7	57 ± 28	484	1.7
250	0.087 ± 0.008	29 ± 6	110 ± 30	946	3.0
500	0.150 ± 0.008	27 ± 5	100 ± 26	859	5.6
2000	N/A	61 ± 17	226 ± 76	2009	N/A
5000	0.068 ± 0.008	69 ± 5	258 ± 52	2314	1.0

MW: molecular weight; N/A: result not available.

tibodies on the SPR probes at the low ng/mL concentration range (Fig. 1). The signal from the serum must be reduced to allow the antigen detection. Even with a reference sensor to account for NSB, NSB on CM-dextran coated sensors is too large to allow a good estimate of the specific binding.

Two different experiments were investigated to monitor NSB. A cold experiment at 0 °C for 14 days was compared to an hour-long experiment at 25 °C. The advantage of the cold experiment is that the kinetics are slower which enables a better estimate of the SPR signal at time = 0. Therefore, a better experiment-to-experiment reproducibility is obtained [31]. The kinetics at 25 °C are faster than the acquisition rate, therefore, a larger error is inherent in the estimate of the initial SPR signal at the higher temperature. A comparison of the variance revealed that the %NSB compared to CM-dextran 500 kDa was not different using the experiment at 25 °C or 0 °C. The absolute value of the NSB will be different since the kinetics and the experiment time are different. In this article, the experiment at 0 °C for 14 days was used to compare the different surface coatings.

3.1.1. Polysaccharides

3.1.1.1. CM-dextrans. The molecular weight of the CM-dextran affects the binding of antigen to antibodies immobilized on the CM-dextran-coated probes, as has been studied previously by Masson et al. [39]. To study this effect, antibodies to cTnI were immobilized on probes that contained CM-dextran with different molecular weights. As shown in the second column of Table 1, the sensor's response to 25 ng/mL cTnI in HBS at pH 7.4 increases with the molecular weight of the CM-dextran layer up to a CM-dextran molecular weight of 500 kDa. Also, a minimum molecular weight of 150 kDa is required to detect the antigen in a 25 ng/mL cTnI solution. CM-dextran 500 kDa is used as a standard because of its commercial availability through the Biacore system.

To study the effect of CM-dextran molecular weight on NSB, NSB with CM-dextran layers ranging from 3 kDa to 5000 kDa was observed in bovine serum with 84 mg/mL total proteins. As mentioned above, NSB is measured as the shift in λ_{SPR} resulting from non-specific binding (reported in nm), or as the shift observed for the system of interest divided by that observed when 500 kDa CM-dextran layers are present on the SPR probes times 100 (reported as %NSB). The binding kinetics for CM-dextran 500 kDa are shown in Fig. 2. As shown in the third column of Table 1, NSB varies from 7 ± 2 nm or 27 ± 9% with 3 kDa CM-dextran to 69 ± 5 nm or 258 ± 52% with 5000 kDa CM-dextran. Low molecular weight CM-dextran (3–75 kDa) layers do not allow the detection of low antigen concentrations, but they are included in this study for the sole purpose of evaluating their effects on NSB. The use of mid-sized CM-dextran, 150 kDa, produced significant levels of NSB; greater than 15 ± 6 nm or 57 ± 28% NSB was obtained compared to 500 kDa. In any case, the NSB signal is 10–100 times greater than the signal from specific binding of 25 ng/mL cTnI or MG; this is especially huge when biologically relevant detection limits of cTnI and MG would be slightly less than 1 ng/mL in human serum. For CM-dextran larger than 500 kDa, the NSB is too great to use in serum.

Coatings must also be compared for both their performance to detect an antigen and their ability to reduce the amount of NSB on the SPR sensor. For that purpose, a per-

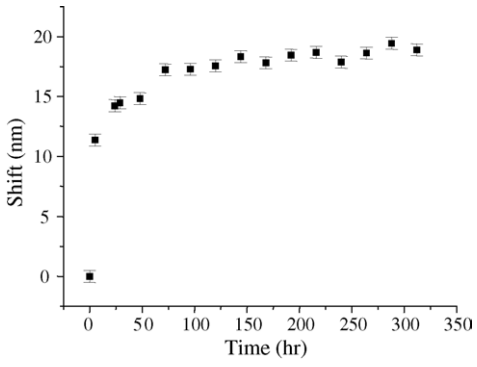

Fig. 2. NSB kinetics for a CM-dextran 500 kDa SPR sensor exposed to bovine serum at 84 mg/mL for 14 days at 0 °C. The Shift is referenced to the first data point acquired when the sensor was exposed to bovine serum. The shift reported in Table 1 is an average of the shift for the final 5 days of the experiment.

formance factor (PF) is described by Eq. (1).

$$\mathrm{PF} = \frac{A_{\mathrm{Shift}}}{\mathrm{NSB}_{\mathrm{Shift}}} \quad (1)$$

where A_{Shift} is the shift from the detection of a 25 ng/mL antigen solution using a given surface coating and $\mathrm{NSB}_{\mathrm{Shift}}$ is the shift recorded in the NSB experiment for the same surface coating. Larger values of PF indicate a better coating, although surface coatings with performance factors less than 1 could be useful if their surfaces are pre-treated with BSA or serum to block NSB sites on the sensor or if the fouling is sufficiently low and stable to be accounted by a reference probe. In this study, coatings showing a PF greater than the 500 kDa CM-dextran reference will be given particular attention. The PFs for different CM-dextran molecular weights are shown in the sixth column of Table 1. These were measured using a 25 ng/mL cTnI solution in HBS pH 7.4. The PF increases from 0 to 5.6×10^{-3} for increasing molecular weights up to 500 kDa. The optimal performance is obtained with CM-dextran 500 kDa. CM-dextran 500 kDa balances a large sensor response and an average NSB performance compared to the other CM-dextran. With larger CM-dextran the performance decreases rapidly. It is explained by a loss in the sensor's response to antigen binding coupled with a large increase in NSB.

The surface coverage in column 5 of Table 1 is an approximation using the calculations from Jung et al. [41] for the thickness of the NSB adsorbed layer and for the calculations of the surface coverage. The thickness (d) of an adsorbed layer can be calculated using Eq. (2). NSB $\mathrm{Shift}_{\mathrm{max}}$ can be calculated from Eq. (3).

$$d = -\frac{l_{\mathrm{d}}}{2} \ln\left(1 - \frac{\text{NSB Shift}}{\text{NSB Shift}_{\mathrm{max}}}\right) \quad (2)$$

$$\text{NSB Shift}_{\mathrm{max}} = m(\eta_{\mathrm{a}} - \eta_{\mathrm{s}}) \quad (3)$$

The value of NSB $\mathrm{Shift}_{\mathrm{max}}$ is obtained knowing the slope (m) of the change in the SPR signal with respect to the refractive index, the refractive index of the adsorbed layer (η_{a}), and the refractive index of the solution (η_{s}). Using the configuration presented here, the slope is 2253 nm/RIU. The refractive index for protein is approximately 1.57 [41]. The penetration depth (l_{d}) of the surface plasmon wave is approximately 230 nm for the wavelength range used in this experiment. The NSB surface coverage (Γ) is calculated from the protein density (ρ) as described by Eq. (4). Protein density is typically 1.3 g/mL [41].

$$\Gamma = d\rho \quad (4)$$

The surface coverage for the CM-dextran layer ranges from 231 ng/cm^2 for CM-dextran 3 kDa to 2314 ng/cm^2 using CM-dextran 5000 kDa. The surface coverage is proportional to the NSB Shift; therefore larger CM-dextran will have larger surface coverage due to larger amounts of non-specifically bound proteins. A monolayer of proteins corresponds to 250 ng/cm^2, therefore, multiple layers are observed.

3.1.1.2. Other polysaccharides. The effectiveness of the polysaccharide coatings at reducing NSB was compared to that observed with 500 kDa CM-dextran (% NSB reported is the NSB for a coating of interest divided by that on CM-dextran 500 kDa times 100). Studies were performed by immersing the probes in bovine serum (84 mg/mL). As shown in Table 2, CM-dextran presented a greater degree of NSB than all the polysaccharide polymers surveyed. The performance of alginic acid is similar to CM-dextran 500 kDa. The NSB decreases from CM-dextran 20 ± 4 nm ($100 \pm 28\%$) = alginic acid 20 ± 2 nm ($97 \pm 22\%$) > CM-hyaluronic acid 9 ± 2 nm ($44 \pm 13\%$) = hyaluronic acid 8 nm (41%, 1 sample). The influence of the surface coatings on the probe sensitivity to antigens was measured based on the probe response when immersed in 25 ng/mL MG. For these studies, anti-MG was immobilized on the probes as described above. CM-hyaluronic acid reduces NSB by 56% compared to 500 kDa CM-dextran, but is only 62% as sensitive in detecting MG compared to 500 kDa CM-dextran supports. CM-hyaluronic performance factor ($\mathrm{PF} = 9.1 \times 10^{-3}$) was only slightly greater than that of CM-dextran ($\mathrm{PF} = 6.6 \times 10^{-3}$). Considering NSB reduction and antigen sensitivity, the overall performance of the sensor is improved by 38% using CM-hyaluronic acid compared to 500 kDa CM-dextran. CM-hyaluronic acid has fewer carboxylic acids on the sugar structure than CM-dextran which explains the lower sensitivity towards MG, but also explains the reduced NSB in serum. Alginic acid has a PF similar to CM-dextran, 6.9×10^{-3} compared to 6.6×10^{-3} for CM-dextran; the 5% improvement in PF is near the noise level of this study. The only coating with a worse performance than CM-dextran was hyaluronic acid ($\mathrm{PF} = 2.5 \times 10^{-3}$) with a 62% regression of PF compared to 500 kDa CM-dextran.

Table 2
Polysaccharides layer performance for MG detection and NSB in bovine serum 84 mg/mL

Layer	MW (kDa)	25 ng/mL MG shift (nm)	NSB Shift (nm)	% NSB compared to CM-dextran 500 kDa	NSB surface coverage (ng/cm^2)	Performance factor ($\times 10^{-3}$)
CM-dextran	500	0.132 ± 0.008	20 ± 4	100 ± 28	643	6.6
CM-hyaluronic acid	>1000	0.082 ± 0.008	9 ± 2	44 ± 13	281	9.1
Hyaluronic acid	>1000	0.020 ± 0.008	8 ($n = 1$)	41 ($n = 1$)	260	2.5
Alginic acid	12–80	0.138 ± 0.008	20 ± 2	97 ± 22	622	6.9

Table 3
Biocompatible layer performance for MG detection and NSB in bovine serum 84 mg/mL

Layer	MW (kDa)	25 ng/mL MG shift (nm)	NSB Shift (nm)	% NSB compared to CM-dextran 500 kDa	NSB surface coverage (ng/cm^2)	Performance factor ($\times 10^{-3}$)
CM-dextran	500	0.132 ± 0.008	20 ± 4	100 ± 28	643	6.6
PMAVA	N/A	0.050 ± 0.008	14 ($n = 1$)	71 ($n = 1$)	456	3.6
Polyacrylic acid	50	0.050 ± 0.008	11 ± 2	50 ± 14	337	4.5
Polylactic acid	330–600	0.056 ± 0.008	8.4 ± 1.1	41 ± 10	264	6.7
Humic acid	2–500	0.041 ± 0.008	7.9 ± 0.7	39 ± 8	249	5.1
OPSS–PEG–NHS	2	0.082 ± 0.008	7.6 ± 0.9	36 ± 8	239	11

3.1.2. Biocompatible polymers

Five biocompatible polymers were investigated to reduce serum NSB. By definition, "biocompatible" polymers do not cause damage or adversely affect biological function when introduced into the body. Therefore in the SPR sensor case, blood coagulation is prevented on the sensor by the biocompatible polymer. As shown in Table 3, all of the biocompatible polymers studied showed reduced NSB compared to CM-dextran. As in Tables 1 and 2 above, performance (NSB%) is measured relative to that observed when CM-dextran 500 kDa is applied. PMAVA sustained only 14 nm shift from NSB (71%, $n = 1$), polyacrylic acid 11 ± 2 nm shift from NSB (50 ± 14%), polylactic acid 8.4 ± 1.1 nm shift from NSB (41 ± 10%), humic acid 7.9 ± 0.7 nm shift from NSB (39 ± 8%), and OPSS–PEG–NHS 7.6 ± 0.9 nm shift from NSB (36 ± 8%). These polymers offer similar anti-NSB performance compared to the polysaccharides. The best biocompatible polymer, OPSS–PEG–NHS, has only a 5% improvement in NSB% compared to the best polysaccharide, hyaluronic acid. This is within the experimental error. Polylactic acid and OPSS–PEG–NHS do not have any carboxylic acids on the backbone. Polylactic acid and OPSS–PEG–NHS are low NSB coatings. The effect of the polymer coatings on probe sensitivity to 25 ng/mL MG was tested using anti-MG immobilized on the polymers. The PFs for most biocompatible polymers are equal to or lower than CM-dextran 500 kDa, except for OPSS–PEG–NHS. OPSS–PEG–NHS has a PF of 11×10^{-3}, 67% greater than CM-dextran, and 20% greater than CM-hyaluronic acid.

3.2. Antigen binding performance

The antigen binding performance was measured with a 25 ng/mL MG or cTnI solution in HBS pH 7.4. CM-dextran and biocompatible polymers performance to detect an antigen have been previously discussed in detail by Masson et al. [30,39]. The shift is used instead of the surface coverage to compare the antibody binding to the sensor because the relative performance is compared between probes, and this is easily correlated using the shift during antibody–antigen binding. The surface coverage does not tell how close to the surface the antibody is bound, nor does it tell whether the antibody is bound in such a matter to maintain activity or accessibility; all of these factors are essential in determining sensor performance. Hence the relative SPR sensitivity may be different than the relative surface coverage. CM-dextran performance increases for molecular weight up to 500 kDa, the maximum shift being 0.150 ± 0.008 nm. The sensor performance was measured using the signal obtained for a 25 ng/mL cTnI solution in HBS at pH 7.4. Biocompatible polymers had different performances for the detection of a 25 ng/mL MG solution in HBS pH 7.4 ranging from 0.020 ± 0.008 nm for hyaluronic acid to 0.138 ± 0.008 nm for alginic acid. In general, the performance was between 0.040 ± 0.008 nm and 0.080 ± 0.008 nm. These were done using a hot antibody reaction.

3.3. Sensor calibrations

Sensor calibrations were performed using MG solutions from 5 to 100 ng/mL in HBS at pH 7.4. Sensors with CM-dextran, OPSS–PEG–NHS, and alginic acid were calibrated. The calibration was linearized using a Langmuir isotherm (Eq. (5)).

$$\frac{1}{\text{Shift}} = \frac{1}{\text{Shift}_{\max} KC} + \frac{1}{\text{Shift}_{\max}} \tag{5}$$

where Shift is the change in the minimum SPR wavelength (nm), $\text{Shift}_{\max}$ the maximum change in the minimum SPR wavelength for a total antigen coverage on the sensor, C the concentration of antigen in solution (ng/mL) and K is the affinity constant for the antigen–antibody system. Every assumption in the Langmuir model is satisfied in our model case. These assumptions are that only one molecule can be adsorbed per site, only one type of site is present, the adsorption of one molecule does not affect the adsorption energy of the other molecules, only one adsorbing species is present, the solution is dilute, and the adsorption is reversible [42]. The reversibility of the adsorption is demonstrated in Fig. 3. The sensors showed different sensitivities to MG. When 1/Shift was plotted as a function of $1/\text{Shift}_{\max}$, the slope of the resulting line provides an estimate of the sensitivity for a given solution concentration of antigen. The greatest sensitivity in the biologically relevant range, 15–30 ng/mL, was for alginic acid followed by CM-dextran, and OPSS–PEG–NHS (Fig. 4 and Table 4). The detection time (maximum binding at equilibrium) is slightly longer for all coatings considered compared to CM-dextran. CM-dextran requires about 5 min to

Table 4
Coating calibration using MG in HBS pH 7.4

Coatings	Sensitivity ((ng/mL)$^{-1}$ nm^{-1})	1/Shift$_{max}$ (nm^{-1})	Concentration range (ng/mL)
Alginic acid	260	1.6	10–50
CM-dextran	113	3.1	10–100
OPSS–PEG–NHS	62	8.7	5–50

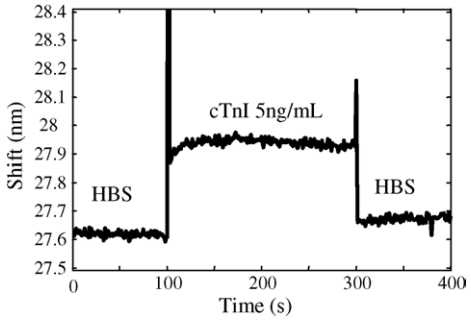

Fig. 3. Measurement cycle HBS–antigen–HBS shows that the sensor is regenerated quickly when placed back in HBS when using low antigen concentration. This case represents a 5 ng/mL cTnI solution in HBS pH 7.4.

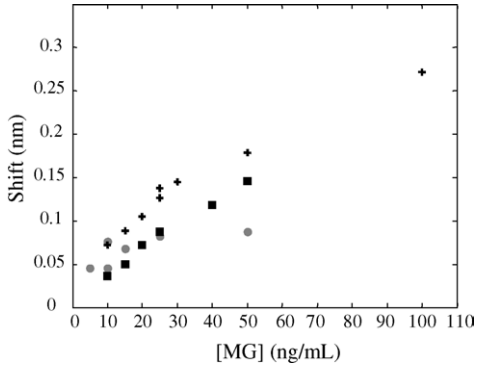

Fig. 4. Langmuir isotherm for alginic acid (black squares), CM-dextran (crosses), and OPSS–PEG–NHS (gray circles). The error on every data point is ± 0.008 nm. The calibration range covers the biologically relevant range (15–30 ng/mL) for MG during myocardial infarctions.

equilibrate while the other coatings require about 10 min. Every coating showed enough sensitivity to detect biologically relevant concentrations of MG. During myocardial ischemia (MIs), MG reaches levels to approximately 15 to 30 ng/mL or higher for serious MI damage.

4. Conclusions

Serum NSB on SPR probes due to the presence of different immobilized species on the probes was reduced by up to 73% using immobilized species other than CM-dextran 500 kDa. Immobilized films from solutions containing CM-dextran 3 kDa gave such performance. However, probes using CM-dextran 3 kDa as an antibody support/anti-NSB layer are unable to detect low concentrations of cTnI. Other polysaccharides decrease the amount of NSB by 59% of that obtained using CM-dextran 500 kDa and they allow MG detection at 25 ng/mL. The use of OPSS–PEG–NHS as an anti-NSB layer offers a slightly better performance (36 ± 8% of the NSB observed for CM-dextran 500 kDa). Every biocompatible polymer studied allowed MG detection at 25 ng/mL. A performance factor is described to compare the surface coatings. A large performance factor exhibits a good sensor's response to detect a 25 ng/mL antigen solution and reduces the amount of NSB. The highest performance factors were obtained using OPSS–PEG–NHS. OPSS–PEG–NHS performance factor was nearly double the one of CM-dextran 500 kDa. Calibration curves were obtained using MG and different coatings to show their performance in HBS pH 7.4. Every coating is able to detect MG at biologically relevant concentrations. Biocompatible polymer can improve the sensors performance compared to CM-dextran. However, many more polymers can be used that could further improve the performance of the SPR sensors. The need to study these polymers is urgent to allow SPR biosensors to be used as a medical diagnostic tool.

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