Reduction of Protein Non-Specific Binding to Various Substrate Materials Using OptiChem® Coatings

Kazunori Emoto, and Michael J. Lochhead
Accelr8 Technology Corporation, 7000 N. Broadway Ste 3-307, Denver, CO 80221

Statement of Purpose: Despite many years of studies, application of hydrophilic polymer coatings is limited due to multiple and tedious coating steps, limited selection of substrates and/or high cost of production. OptiChem® is a novel formulation of poly(ethylene glycol) derivatives mixed with surfactant, surface binding and cross-linking reagents (U.S. Patents 6,844,028 and 7,067,194). The simple coating procedure provides extremely low non-specific protein adsorption and facilitates specific interaction of tethered ligands and reporter groups with analytes. In this report, we present applicability of OptiChem® coatings to a variety of substrate materials and formats and demonstrate low non-specific protein adsorption, as well as enhanced specific interaction of tethered ligands with biomolecules and organisms.

Methods: Hydrogel formulation was prepared by mixing difunctional or monomethoxy-monofunctional PEG succinimidylcarboxypentyl NHS ester (MW 3400 and 5000, respectively) with (3-trimethoxysilylpropyl) diethylenetriamine and 6-azidosulfonylhexyltriethoxy silane and polyoxyethylene sorbitan tetraoleate in dimethylacetamide and/or dimethylsulfoxide. Spin coating was used to apply the formulation to flat substrates, including glass slides, indium tin oxide (ITO) coated glass slides, silicon wafers, and tissue culture polystyrene dishes. Solution-phase deposition was used to coat polypropylene centrifuge tubes. To test their ability to resist protein adsorption, the NHS ester groups was converted to methoxy group. Non-specific protein adsorption was demonstrated by using ELISA-like method. Samples were exposed to solutions of human fibrinogen (100, 10, 1 and 0µg/mL) at 22°C for 1 hour, anti-fibrinogen-horseradish peroxide (HRP) conjugate (5µg/mL) for 1 hour, and 1-component TMB. The reacted TMB was transferred to microplate and the absorbance of the solution was measured by microplate reader. Control surfaces consisted of bare (untreated) substrates and substrates blocked with bovine serum albumin (BSA). In addition to the ELISA method, fluorescently labeled goat serum was also used for direct measurement of protein adsorption (data not shown).

Results/Discussion: Figure 1 shows fibrinogen non-specific binding performance on coated glass and tissue culture polystyrene substrates. High signal in 0µg/mL fibrinogen for untreated samples was a result of high non-specific adsorption of anti-fibrinogen-HRP. This indicates that bare materials strongly attracted proteins. Blocking of bare surface with bovine serum albumin is widely used to prevent non-specific adsorption in the field of bioanalysis. BSA blocking reduced non-specific adsorption appreciably, and adsorption of anti-fibrinogen-HRP to the BSA-blocked surfaces is negligible. OptiChem coatings reduced non-specific adsorption significantly. Adsorption of Alexa555-labeled goat serum showed only 1/100 of fluorescence compared with untreated materials.

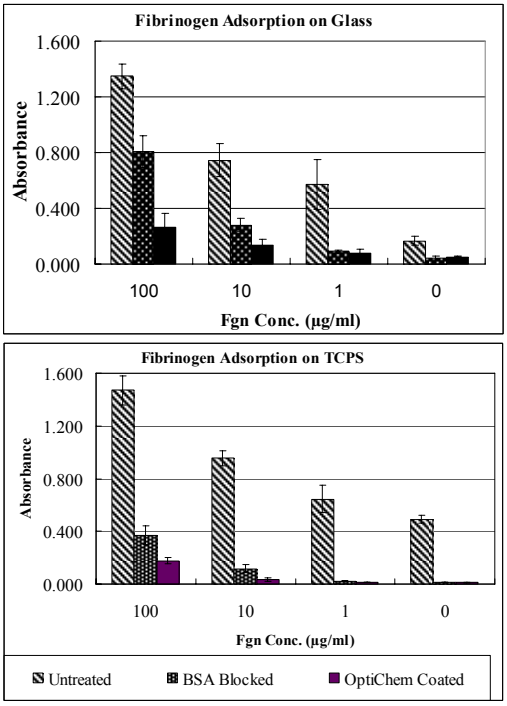

Figure 1. Fibrinogen non-specific binding (NSB) to uncoated, BSA-blocked, and OptiChem coated subsrates. NSB is reported in absorbance units of the colorimetric peroxidase substrate (TMB). Bars represent the average of two separate wells on three substrates (six sites total). Error bars are one standard deviation.

OptiChem was also successfully applied polypropylene centrifuge tubes by a simple solution-phase deposition method after first priming the plastic surface with a air plasma treatment. Non-specific adsorption of IgG-HRP (10µg/mL) was reduced by 1/20 compared with untreated and plasma-treated PP tubes.

Specific protein binding was demonstrated by the covalent attachment of streptavidin to the NHS-activated coatings. Specifically attached streptavidin provided a > 500:1 biotin binding activity relative to a no-streptavidin OptiChem control, as assayed by biotin-bovine serum albumin-Alexa555 with fluorescence detection.

Conclusions: OptiChem formulation was successfully coated on a variety of substrates including glass, metal oxide and plastics. The coatings reduced non-specific adsorption of proteins and could be modified to provide specific molecule attachment. OptiChem is unique among PEG-based coatings in the ease of application and substrate versatility.