Generalized Platform for Antibody Detection Immunosensor

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Primary CNF Tools Used: ABM contact aligner, MVD 100

Abstract:

Immunosensors are diagnostic devices that convert specific antigen-antibody interactions, by means of a transducer, into an electrical signal. Immunosensors are very efficient, simple, affordable, and cost-effective point-of-care systems for medical diagnosis. Our study focuses on the use of polymer chemistry and photolithography to create a unique antibody detection platform. This immunosensor takes advantage of the antibody catalyzed water oxidation pathway (ACWOP) process, in which all antibodies catalyze the production of hydrogen peroxide that can be detected using a colorimetric assay. This report focuses on the fabrication of the platform that make up our immunosensor device.

Summary of Research:

Pandemic infectious diseases have affected many people throughout history, sometimes killing millions of people such as the Plague in 14th century Europe, and the flu. One of the most common methods of detecting such diseases is through antibody detection using a serological assay. In our group, Welch, et al., in 2014 developed an immunobiosensor based on patterned polymer brushes on gold, which demonstrated extremely high sensitivity (2pM) for the specific antibody [1]. This design took advantage of the excellent properties of poly(oligo(ethylene glycol) methacrylate) (POEGMA) polymer brushes by preventing non-specific adsorption and allowing the anchoring of the functional haptens [2].

With that in mind, we would like to improve this sensor by using colorimetric detection as the electrochemical signal in the original design was not as intuitive and required additional parameters complicating our immunosensor. This sensor consists of patterned poly (oligoethylene glycol methacrylate) (POEGMA) polymer brushes on a glass substrate that were polymerized using atom transfer radical polymerization (ATRP), surrounded by a photosensitizer that was functionalized onto the surface of the silica platform.

Photosensitizer is responsible for the production of singlet oxygen reacting with water to produce hydrogen peroxide. In the past, our group used a ruthenium-based photosensitizer that was electropolymerized on a gold surface. To enable an economic colorimetric design, we selected to utilize Rose Bengal rather than Ru(4-vinyl-4′-methyl-2,2′-bipyridine)3]$^{+2}$ as photosensitizer due to its high quantum yield (0.75)[3], low price and ease of anchoring.

The detection mechanism is based on antibody catalyzed water oxidation pathway (ACWOP)[4], which is the same as previous work in our group as shown in Figure 1.

The dimension requirement for the sensor is well above the micron level (150 μm line pattern), which could be easily achieved with 365 nm UV photolithography using the ABM contact aligner. The bottom-up patterning process suits this application better for the resulting structural integrity. Since we already know that the monolayer made of silane compounds is unable to completely block all the reactive sites on the silicon substrates, deposition of the second chemical species must be conducted while the photoresist is still present on the surface, otherwise, the mixed chemical species will show up in the pre-defined area.

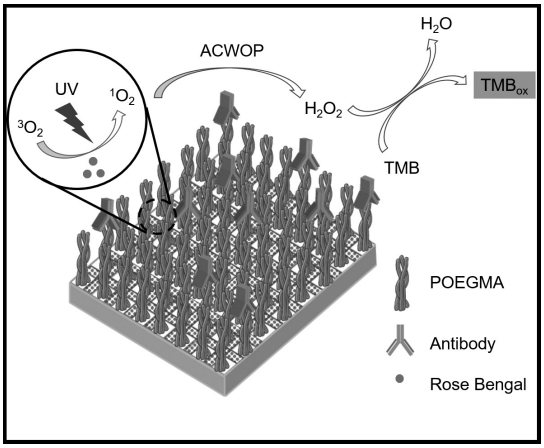

Figure 1: Detection mechanism for the proposed immunosensor. Under UV light exposure, singlet oxygen is formed by exciting Rose Bengal. Hydrogen peroxide is then produced through ACWOP. Hydrogen peroxide subsequently oxides TMB resulting in a change from clear to blue color for colorimetric readout. TMB: 3,3′,5,5′ tetramethylbenzidine.

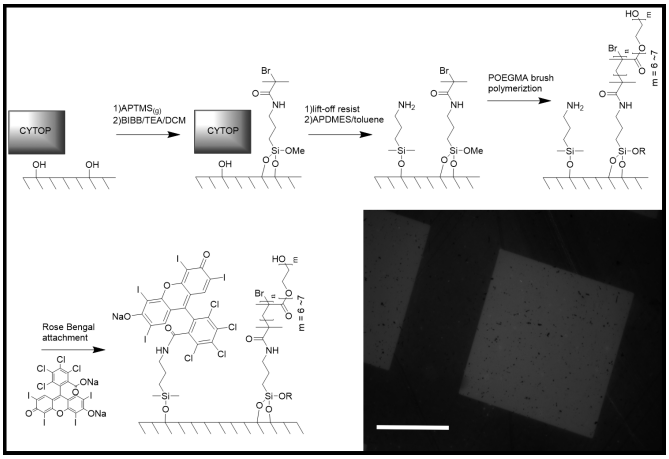

Figure 2: Process flow for the fabrication of the biosensor platform and the picture of the platform taken with a fluorescent microscope.

The CYTOP bottom-up patterning process was then applied to fabricate the functional surface. The fabrication process and results are shown in Figure 2. The 3-aminopropyltrimethoxysilane (APTMS) must be deposited before polymerization since it could also react with the hydroxy group on the POEGMA polymer brushes. This surface functionalization was done using the molecular vapor deposition tool (MVD100). However, it turned out that the Rose Bengal could still adsorb on the surface with POEGMA polymer brushes even though the amount is limited. This happened because an esterification reaction used for anchoring Rose Bengal on an amine could also anchor it on a hydroxy group.

To create more defined patterns, in the future other orthogonal reactions should be selected to anchor the compounds onto the surface. For example, 3-mercaptopropyltrimethoxysilane could be used instead of the APTMS to be the anchoring group, which could be reacted by a thiol-ene click reaction. Another issue that was encountered was that the Rose Bengal monolayer on the surface is not enough to trigger significant signal. Such problems may be further solved by using polymer brushes on the surface to provide more surface area for the anchoring of the Rose Bengal. If the thickness of the polymer brushes is high enough, the amount of the Rose Bengal could be increased to more than 100 times the amount grafted on the surface and this should be enough to create detectable signal. With the orthogonal anchoring chemistry for the functional groups and polymer brushes for Rose Bengal anchoring, this platform could be more applicable for fabrication and provide easy detection by colorimetric methods.

In conclusion, a CYTOP patterning process could be easily extended to other fields and enable better complex platform fabrication.

References:

[1] Welch, M. E., et al. Generalized Platform for Antibody Detection using the Antibody Catalyzed Water Oxidation Pathway. J. Am. Chem. Soc. 136, 1879-1883 (2014).

[2] Harder, P., Grunze, M., Dahint, R., Whitesides, G. M. and Laibinis, P. E. Molecular Conformation in Oligo(ethylene glycol)-Terminated Self-Assembled Monolayers on Gold and Silver Surfaces Determines Their Ability To Resist Protein Adsorption. J. Phys. Chem. B 102, 426-436 (1998).

[3] DeRosa, M. Photosensitized singlet oxygen and its applications. Coord. Chem. Rev. 233-234, 351-371 (2002).

[4] Wentworth Jr., P. Antibody Catalysis of the Oxidation of Water. Science 293, 1806-1811 (2001).