Nanoparticles Based Immunosensors for Detection of Biomarkers

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Sensitive quantitative detection of disease-related proteins is critical to many areas of modern biochemical and biomedical research. In particular, the clinical measurement of cancer biomarkers shows great promise for early disease detection and highly reliable predictions. For point-of-care applications, the sensors need to be inexpensive, operationally simple, and highly sensitive to address both levels of the biomarkers in normal and cancer patient serums. Electrochemical immunoassay has attracted considerable interest because of its intrinsic advantages such as portability, low cost and high sensitivity. In order to meet the increasing demand for early and ultrasensitive detection of biomarkers, various signal amplification technologies have been developed. One of the most popular strategies is enzyme-functionalized nanoparticles used as tracer to enhance the detection sensitivity by loading a large amount of enzymes toward a sandwich immunological reaction event. In our group, various nanomaterils have been used as carrier to load enzymes and antibodies including graphene, carbon nanotubes (CNTs), carbon nanospheres, gold nanoparticles, silica nanoparticles and carboxylated magnetic beads.

We proposed a novel electrochemical immunosensor for sensitive detection of cancer biomarker α-fetoprotein (AFP) using a graphene sheets sensor platform and functionalized carbon nanospheres (CNS) labeling with horseradish peroxidase-secondary antibodies (HRP-Ab2). Porous CNS displayed unique advantages owing to the tenability of particle size and shape as well as the resident porosity that promotes diffusion of guest molecules through interconnected micropores. A “green” synthetic approach has been developed that involves the transformation of sugars into homogeneous and stable colloidal CNS, which is hydrophilic. Such surface-functionalized CNS and porous structures are potentially beneficial for labeling and electrochemical processes. Greatly enhanced sensitivity for the cancer biomarker is based on dual signal amplification: First, the synthesized CNS yielded homogeneous and narrow size distribution, which allowed several binding events of HRP-Ab2 on each nanosphere. Enhanced sensitivity was achieved by introducing the multi-bioconjugates of HRP-Ab2-CNS onto the electrode surface through “sandwich” immunoreactions. Secondly, functionalized graphene sheets used for the biosensor platform increased the surface area to capture a large amount of primary antibodies (Ab1), thus amplifying the detection response. On the basis of the dual signal amplification of graphene sheets and the multi-enzyme labeling strategy, the developed immunosensor showed a 7-fold increase in detection signal compared to the immunosensor without graphene modification and CNS labeling.

In our group, a novel electrochemical immunosensor for the detection of tumor necrosis factor-alpha (TNF-α) based on poly-(guanine)-functionalized silica nanoparticles (NPs) label has been presented. The detection of mouse TNF-α via immunological reaction is based on a dual signal amplification: (1) a large amount of guanine residues introduced on the electrode surface through sandwich immunoreactions and poly(guanine)-functionalized silica NP label; (2) $Ru(bpy)3^{2+}$-induced catalytic oxidation of guanine, which results in great enhancement of anodic current. The poly[G]- and avidin-functionalized silica NP label was prepared by covalent binding poly[G] and avidin to the silica NP surface using the conventional coupling reagent EDC and NHS. Briefly, carboxylic acid functionalized silica NPs were prepared from amino-modified silica NPs with glutaric anhydride in DMF and activated with EDC and NHS in an aqueous solution to obtain NHS ester-terminated silica NPs. The activated silica NPs were then reacted with avidin and amino-modified poly[G]. The functionalized silica NPs were separated from excess reactants by centrifuge. The amount of guanine per silica NP was determined with chronocoulometry by measuring the charges from the guanine catalytic oxidation at the SPE, which was immobilized with the functionalized silica. It was found that there are ~60±10 strands of $poly[G]_{20}$ per silica NP. Accordingly, the average surface coverage of $poly[G]_{20}$ on a silica surface was determined to be ~8.5 □× 10^{12} molecules cm^{-2}, which is comparable with that of oligonucleotides reported on other planar substrates, e.g., glass and silicon. The synthesized silica NP conjugates were characterized with atomic force microscopy, X-ray photoelectron spectroscopy, and electrochemistry. The performance of the electrochemical immunosensor was evaluated and some experiment parameters (e.g., concentration of $Ru(bpy)_3^{2+}$, incubation time of TNF-α, etc.) were optimized. The detection limit for TNF-α is found to be 5.0x 10^{-11} g mL^{-1} (2.0pM), which corresponds to 60 amol of TNF-α in 30 μL of sample. This immunosensor based on the poly(guanine)-functionalized silica NP label offers great promise for rapid, simple, cost-effective analysis of biological samples.

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