**Effect of Functionalization Method on the Sensing Performance of 3D Nanostructured Electrodes**

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**Statement of Purpose:** Electrochemical based biosensors offer a cost-effective and more specific means to measure the electrical responses resulting from biochemical interactions between the sensitive probe molecules and target analytes. In such a biosensor, the sensing performance is related to the surface area of its electrode because a large surface area is beneficial not only for enzyme immobilization but also for electron transfer. The surface area of an electrode can be increased by using nanostructures. However, since most nanostructures are made of inorganic materials, they have to be functionalized for biorecognition purposes if they were to be used as electrodes. In many situations, biosensitive molecules cannot be immobilized directly onto the surface of most inorganic materials, thus anchoring molecules are necessary. Therefore, the ability to improve the performance of these inorganic-based nanostructured electrodes relies not only on the morphological design of the nanostructures but also on their functionalization methods.

**Methods:** Starting with 3D skyscraper gold nanostructures fabricated on glass substrates (see Figure 1b-inset), we use three different functionalization methods to immobilize glucose oxidase (GOx) onto these electrodes. In the first two methods, we used two self assembled monolayer (SAM) alkanethiols: 1) 3-mercaptopropionic acid (MPA): HS-$(CH_2)_2$-COOH and 2) 11-mercaptoundecanoic acid (MUA): HS-$(CH_2)_{10}$-COOH. In the third method, we used conducting polymer, polypyrrole. For the two SAM cases, the 3D nanostructures were immersed in ethanol solution containing 10 mM of either the MPA or MUA molecules for 24 hours. The SAM modified surfaces were rinsed in 75% ethanol and immersed in a 0.1M 2-(N-morpholino) ethanesulfonic buffer solution (pH of 3.5) containing 2mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and 5 mM N-hydroxysuccinimide for activation for two hours. After washing in PBS, the activated electrodes were placed in PBS solution at pH 7.4 containing 1 mg/ml of GOx for two hours under constant stirring for maximum enzyme immobilization. For the polypyrrole (PPY) case, a galvanostatic electrodeposition process was performed in 0.1 M KCl solution containing 0.05 M pyrrole and 0.5 mg/ml GOx to form a porous film on the electrode surfaces. This process traps GOx inside the porous structure of GOx/PPY.

These functionalized electrodes were then used for GOx catalyzed glucose detection. Amperometric measurements were made in PBS containing 3 mM p-benzoquinone and certain amount of glucose at a constant electrode potential of 0.35 V (vs. Ag/AgCl). During amperometric experiments, the solution was stirred constantly for ensuring instant equilibrium for mass transport. During each test run, the background current was allowed to stabilize before a drop of glucose was added to the solution. After the amperometric current response reached a steady state, another drop of glucose was added and the corresponding current response was measured until a new steady state was reached.

**Results:** From three repeated runs of amperometric experiments, we first constructed a calibration curve for each functionalization case by plotting the current value against glucose concentration. Figure 1a shows the calibration curve for the PPY case. From these calibration curves, we determined the sensitivity values: 6.34, 3.08 and 2.72 ($\mu A/cm^2/mM$) for the PPY, MPA and MUA cases, respectively, as shown in the bar graph in Figure 1b.

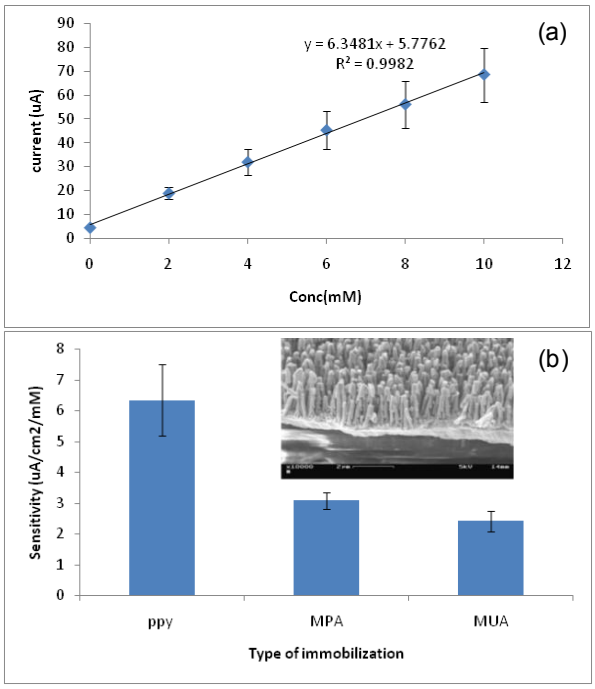


Figure 1. (a) Calibration curve for the PPy case; (b) Sensitivity values for the three cases.

**Discussions & Conclusions:** Using 3D electrodes having skyscraper nanopillar structures as electrodes for glucose detection, we showed that the sensing performance of these inorganic electrodes is indeed affected by the functionalization methods. The PPY case showed the highest detection sensitivity followed by the MPA case, and the MUA case showed the lowest sensitivity. Since MUA is a longer chain alkanethiol than MPA, it is expected to block more electron transfer than MPA. These results suggest that using physical entrapment of GOx near the electrode surface within a porous PPY structure may provide a more efficient means for facilitating enzymatic reaction and electron transfer. By contrast, using SAM layers (i.e., the MPA, MUA) to tether GOx may pose a barrier to electron transfer across the electrode/electrolyte interface, thus lowering the detection sensitivity.