Glucose detection at films composed of Ir oxide nanoparticles on carbon supports

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Diabetes is an increasingly widespread disease, resulting from the inability of the body to properly produce or utilize the hormone insulin, which maintains normal blood glucose levels (BGLs). Glucose biosensors are used to monitor BGLs, in order to prevent the medical complications that result from inappropriate levels. There are many different types of glucose biosensors currently being developed. However, they suffer from a range of problems, including slow kinetics and a dependence on O_2, which contributes to poor sensor reproducibility.

This work involves the optimization of an amperometric glucose biosensor, composed of Ir oxide nanoparticles (IrOx NPs), glucose oxidase (GOx), and Nafion[1,2]. IrOx is an excellent sensor matrix material, as it is conductive, robust, porous, and biocompatible. GOx is required for high selectivity, as it only reacts with β-D-glucose. Nafion has a two-fold function in the film, as it acts as both a binding agent and a repellent of negatively charged electroactive interfering species present in blood, such as ascorbic acid or uric acid.

The specific objectives of this work are to fabricate a biocompatible, third-generation glucose biosensor that is robust, sensitive, and responds reproducibly to glucose, independent of the concentration of O_2 in the sample. In this work, films were fabricated by immobilizing GOx on sputtered Au (on glass), as well as carbon substrates of a range of pore diameters.

To immobilize GOx, diazonium salt chemistry was used to first deposit multilayers of nitrobenzene on the substrate. The nitro groups were then electrochemically reduced to amines[3,4] and a succinic anhydride linker was attached via an amide bond to the amino phenyl groups. EDC and NHS, two common carboxylic activating compounds, were then used to ensure efficient amide bond formation between the tethered –COOH groups and the amino acids that comprise GOx[4]. Ir NPs (in the form of an Ir sol), mixed with Nafion, were then deposited around the immobilized enzymes and electrochemically oxidized to IrOx. This formed an interconnected and electronically conductive matrix of IrOx NPs for direct electron transfer from the active site of GOx to the underlying current-collecting substrate. Glucose testing experiments were performed by stirring 20 µL of 1.0 M glucose into the phosphate buffer solution (25 mL) in which the sensor was submerged.

In the presence of O_2, the active site of GOx reacts with glucose to produce hydrogen peroxide, which is then detected electrochemically at the electrode materials. This method of detection is not optimal as, in practice, blood O_2 levels tend to fluctuate and the accumulation of H_2O_2 is not desirable. When the IrOx NPs are close enough to the active site, however, they can directly mediate electron transfer from GOx to Au at a more rapid rate than does O_2[1,2]. If all of the GOx molecules are in good contact with the IrOx network, electron transfer should occur predominately through the IrOx network, resulting in an O_2-independent glucose sensor.

The immobilization of GOx to the Au substrate and the subsequent deposition of IrOx matrix around the deposited GOx ensures that the IrOx NPs are in close contact with the buried active site so that electron tunneling can take place. This fabrication technique produces films that are approaching O_2-independence and also exhibit good reproducibility (<15% standard deviation). However, the current signal is low (ca. 4 $\mu A/cm^2$) in comparison to what is observed with other glucose sensors, including our own (> 40 $\mu A/cm^2$) in a deaerated environment[1,2], owing to the small number of GOx molecules immobilized in the sensing film (ca. 10 times less).

To enhance the glucose signal, the real substrate surface area was increased so that the quantity of immobilized GOx could be significantly enhanced. High surface area carbon inks[5,6] were deposited and dried on a Au substrate, and GOx was then immobilized on the films, as usual. It was found that the carbon films could not withstand the organic solvent required for the electrochemical diazonium salt reduction and the attachment of the succinic anhydride linker. As such, the diazonium salt reduction was performed in an aqueous solution of the same salt concentration (1.0 mM). It was found that the nitrophenyl surface coverage could be increased, although in the absence of the succinic anhydride linker, the degree of GOx immobilization was inadequate and the glucose signals, while O_2-independent, remained small (<2 $\mu A/cm^2$). In order to attach the linker to the aminophenyl groups without dissolving the high surface area carbon support, the nitrobenzene groups were chemically reduced [5] in situ, followed by the attachment of the succinic anhydride linker and GOx. The solution was then mixed with the Ir sol and the resulting ink was deposited on the sputtered Au substrate to form a reproducible matrix for direct electron transfer (O_2-independent) from the enzyme active site to the underlying Au substrate.

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