Optimization of a Capacitive Sensing Organic Electrochemical Transistor Immunoassay

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Introduction:

The development of fast, accurate and sensitive immunoassays is of major importance for the early detection of several diseases. Traditional methods, such as the ELISA and Western Blot, operate on the time scale of hours to days; we thus aim to integrate organic electrochemical transistor (OECT) arrays with traditional immunoassays to provide both the molecular specificity of an immunoassay and the speed of a microelectronic system. Previous work in this field has been based on the integration of external enzymes [1] or on complex fabrication methods[2]. We herein present initial results of a simple capacitive OECT sensing platform for direct immunodetection.

The sensing principle was based on the capacitive change caused by the antigen-antibody affinity binding to the biofunctionalized poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) transistor channel. When molecules are immobilized onto the channel, they add a resistive and capacitive element to the equivalent circuit of the OECT. Accordingly, the impedance changes due to the added mass to the channel, and a complex combination of the induced electrostatic charges of the added molecules is reflected in a shift in the transconductance. A schematic representation of the OECT along with a the simplified equivalent circuit, and the transistor characteristic curves depicted in Figure 1.

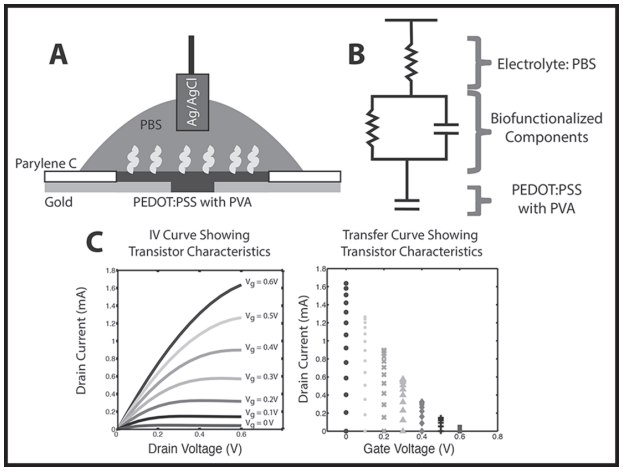

Figure 1: A] Schematic representation of the OECT. B] design of a simplified equivalent circuit. C] Steady state characteristics: output and transfer curves of the fabricated OECTs

Experimental Methods:

Gold electrodes were patterned onto glass slides via photolithography and chemical vapor deposition. A layer of Parylene-C was also deposited through vapor deposition, followed by a layer of 2% MicroClean soap solution followed by a sacrificial layer of Parylene-C, The last part of the fabrication process involved the development of photolithographically patterned channels, the dimensions of which were 10 × 10 μm^2. Finally, the (semi)conducting solution was spin-coated to the channel, and the gate that were exposed via reactive ion etching.

In order to introduce hydroxyl groups onto the transistor channel, a PEDOT:PSS:polyvinyl alcohol (PVA) blended solution was used as the active solution [3]. Following, the channel was further functionalized and treated with a (3-glycidyloxypropyl) trimethoxysilane (GOPS) solution in order to introduce epoxy groups on top of the channel and thus allow protein immobilization.

Initially, in order to standardize our OECT based immunoassay, a model system was employed, consisting of a primary and a secondary (fluorescent) antibody. The primary antibody was primarily immobilized onto the epoxy-modified transistor channel, and a bovine serum albumin (BSA) solution was deposited prior to the immobilization of the secondary antibody, in order to avoid unspecific binding. All the aforementioned steps, including the biofunctionalization of the channel are schematically represented in Figure 2.

Results and Discussion:

We herein fabricated PEDOT:PSS-based OECT towards the development of an immunosensing platform, Initially our work involved the study of, different biofunctionalization methodologies. It was found that the thicker the GOPS layer onto the transistor channel, the higher the standard

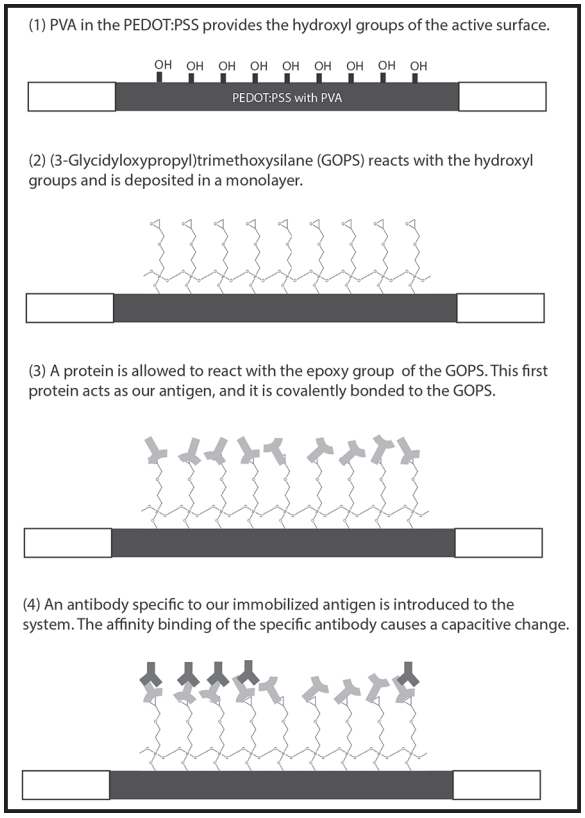

Figure 2: Biofunctionalization concept diagram.

deviations were observed leading to less consistent data, thus indicating the necessity of developing a GOPS mono-layer deposition method. The biofunctionalization methodology was validated by checking the fluorescence of the device after the immobilization of the secondary antibody.

Additionally, we studied the effect of the electrolyte (PBS) concentration on the transconductance cut-off frequency, It was found that the transconductance cutoff frequency decreases as the PBS concentration decreases, due to the electrolyte's equivalent resistance in the equivalent circuit. Given that the theoretical transconductance cutoff is dependent on the combined impedance of all the elements in the circuit and in order to be able to sense small changes in impedance, the OECTs were tested after each critical immobilization step with varying PBS concentrations (i.e., 1X, 0.1X, 0.01X, and 0.001X). Those results are graphed and shown Figure 3. Ultimately, none of the higher electrolyte concentrations (1X, 0.01X, and 0.01X) show clear shifts between steps in the biofunctionalization procedure; the trendlines have large error bars that all overlap giving null results. The OECT tested under PBS 0.001X, however, shows promising results, as there are clear, distinguishable transconductance shifts for the covalent bonding of GOPS to the channel and for the covalent bonding of the first antibody to the GOPS. The lack of shift in subsequent steps for the PBS 0.001X sample makes sense if the secondary antibody is not binding or is currently too small to detect. To validate that the normalized shifted curves are an accurate representation of the transconductance cutoff relationship, the derivative functions were graphed and yielded the same results as the visual shifts.

These results indicate that the capacitive method can successfully detect covalent bonding onto the transistor channel.

Future Work:

The next step of this work is to successfully detect the antigen-antibody affinity binding via a transconductance shift. We plan to improve the biofunctionalization process by focusing on the GOPS monolayer formation onto the transistor channel, since variations in a thick GOPS layer interfere creates noise in the transconductance that obscures the desired shift. Moreover specific disease detection models will be also studied.

Acknowledgements:

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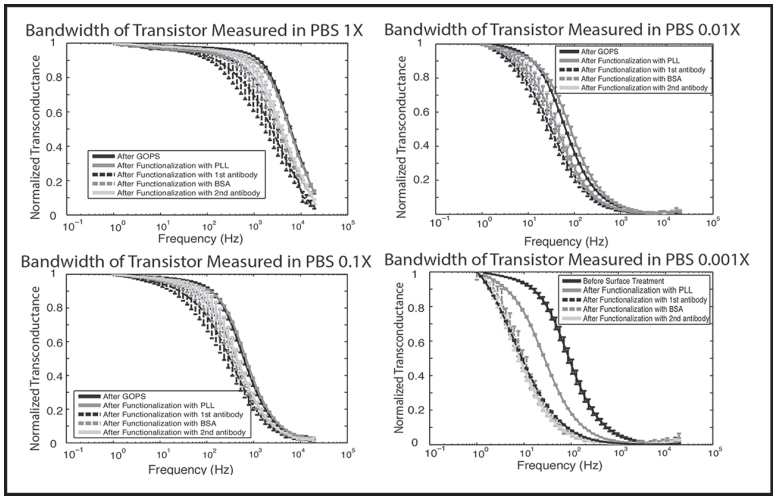

Figure 3: Frequency dependence of the transconductance in varying PBS concentrations between all the critical immobilization steps.