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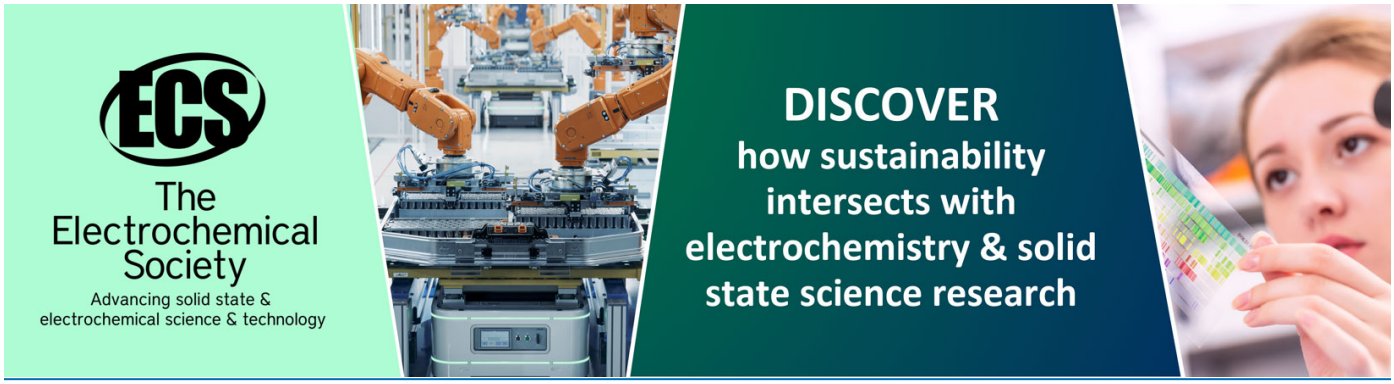

Virus-assembled technology for next generation bioenergy harvesting devices

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Abstract. This work reports Tobacco mosaic virus (TMV)-based assembly of glucose oxidase (GOx) electrodes for the development of advanced enzymatic biofuel cells (EBCs). Cysteine-modified TMV (TMV1cys), self-assembled on Au electrodes, has served as a template for augmenting the GOx immobilization density via a robust chemical conjugation route. Enhanced enzymatic activity of the TMV1cys/GOx electrodes have been confirmed via colorimetric assay, and the electrochemical transduction of the catalytic reactions via mediated electron transfer resulted in a ~25-fold increase in electrochemical current compared to previous work using a similar strategy. This enhancement is attributed to the on-chip bioconjugation strategy presented in this work, leveraging the robust and high density self-assembly of TMV1cys on Au surface. The performance of an EBC consisting of TMV-assembled enzyme electrodes is reported for the first time, generating a maximum power density of 860 nW/cm^2.

1. Introduction

Enzymatic biofuel cells (EBCs) have received increasing attention from a broad spectrum of researchers due to (1) their exciting potentials for sustainable energy harvesting from biofuels (e.g. glucose, ethanol, cholesterol), and (2) further opportunities to autonomously operate wearable and implanted devices with fuel sourced from body fluids [1]. However, despite the numerous enabling features, including biocompatibility, high-specificity, and renewability, the low current/power density has been one of the major bottlenecks limiting their practical implementation. Recent approaches demonstrating enhanced EBC performances via use of nanoscale materials (e.g. carbon nanotubes, Au nanoparticles) provides a potential route - increasing enzyme reaction density and its electrode interface - for the technological development of EBCs in real-world applications [2].

In this work, genetically-engineered Tobacco mosaic virus (TMV) has been utilized as a robust molecular assembler for high-density immobilization of glucose oxidase (GOx) on electrodes. TMV is

a plant virus featuring a rod-like nanostructure (300 nm length, 18 nm diameter) with thousands of functional receptors uniformly arranged on its surface. This versatile biomacromolecule has been utilized in a wide range of nanotechnology areas including drug-delivery, vaccine development, and energy storage devices [3]. The results presented here demonstrate TMV as a high-density molecular assembly template for GOx immobilization on electrodes, and provide a fundamental understanding behind the electrochemical conversion mechanism of the virus-assembled electrode system driving the development of next-generation EBCs.

2. Materials and methods

2.1. Virus-based GOx-electrode assembly

Figure 1 describes the TMV-based on-chip bioconjugation process developed in this work for high-density GOx immobilization. Au electrodes were prepared on a Si wafer with 500 nm PECVD of SiO_2 followed by sputtering of 30/150 nm Cr/Au. Each conjugation step lasted 5 hours at room temperature inside a humid chamber. The electrode surface was rinsed 3X in 0.1 M PBS (pH7.2) after each step to remove any unreacted/excess molecules.

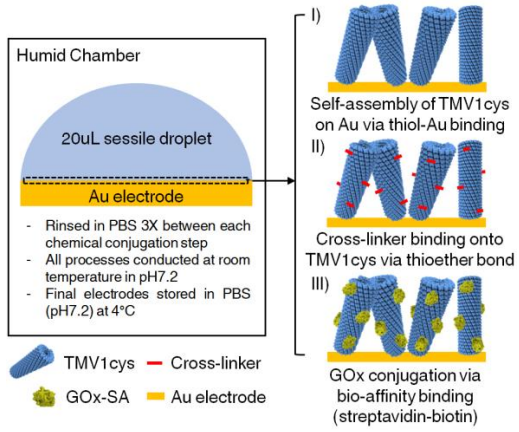

Figure 1. Illustrations outlining the on-chip TMV-GOx bioconjugation process: I) self-assembly of TMV1cys on Au surface via thiol-Au binding by introducing 20 µL of TMV1cys solution (0.2 mg/ml in 0.1 M PBS, pH7.2), covering ~7 mm^2. The electrode sample was incubated overnight at room temperature as described in previous work [4]. This was followed by successive introductions of II) a cross-linker (CL, EZ-Link^{TM} maleimide-PEG_{11}-biotin, Fitzgerald Industries International) and III) streptavidin-conjugated GOx (GOx-SA, 65R-S124, Thermo Fisher Scientific)

2.2. Electrode Characterization

A colorimetric assay was performed to confirm enzymatic activity of GOx immobilized via TMV1cys. Hydrogen peroxide is a byproduct of glucose oxidation catalyzed by GOx and can be measured by introducing a reporter enzyme, specifically horseradish peroxidase (HRP, Alpha Aesar) with a chromogenic substrate. Luminol (Fisher Scientific) was used as the chromogenic substrate in this work; the reduced product of the luminol, 3-aminophtalic acid, results in a yellowish color change of the solution and the optical absorption at $\lambda = 390$ nm was correlated with the level of the enzymatic reaction.

The electrochemical characterization was carried out using a benchtop potentiostat (VSP-300, Biologic) in a beaker-level testing set-up with a three-electrode configuration (reference: Ag/AgCl, counter: Pt, working: sample electrodes) in 0.1 M pH7.2 PBS. Cyclic voltammetry (CV) measurements were conducted at a 5 mV/s scan-rate within a potential window of -0.5 – 0.7 V. For biofuel cell characterization, the sample electrodes (working) were assembled with a Pt-cathode (counter/reference) in a two-electrode configuration. The open-circuit-potential (OCP) was monitored when connected to different resistive loads to evaluate power performance.

3. Results and analysis

3.1. Enhanced enzyme density with TMV-based on-chip conjugation

The colorimetric assay was performed with six different electrodes (figure 2). The negative controls (E1-E3) showed no relative change in optical density compared to a baseline measured without any electrodes immersed. Compared to the positive controls injected with GOx (E4, E5), the GOx

immobilized onto the Au surface via TMV1cys (E6) showed a higher colorimetric response resulting in higher optical density: a ~2-fold increase compared to the positive controls. This confirms that the TMV1cys and the chemical conjugation were effective for robust and high-density immobilization compared to the non-specifically bound GOx samples.

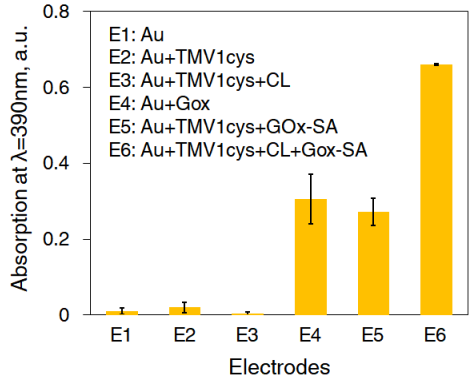

Figure 2. Characterization of GOx immobilization via absorption spectroscopy using H_2O_2/luminol/horseradish peroxidase (HRP) based colorimetric assay. Increased GOx immobilization with TMV1cys and CL are confirmed with increased absorption intensity at λ = 390nm (N=3).

3.2. Electrochemical conversion kinetics and performance

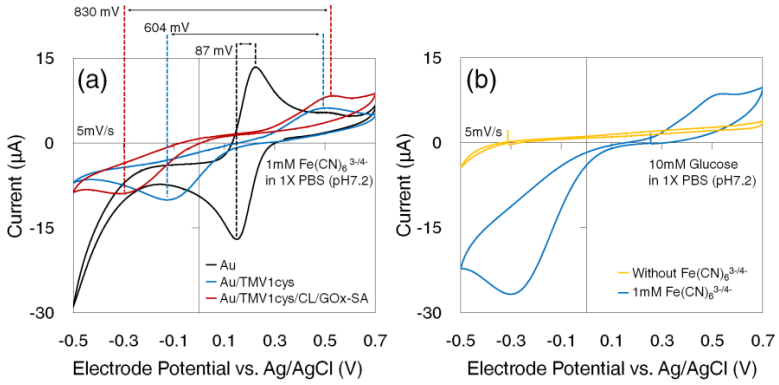

Figure 3. Electrochemical characterization showing (a) a change in charge transfer kinetics with electrode functionalization and (b) effective charge transfer mechanism for the Au/TMV1cys/CL/GOx-SA electrodes.

Electrochemical characterization was performed to understand the impact of the on-chip bioconjugation on the electrode kinetics and to identify an effective charge transfer mechanism for the electrochemical conversion of the enzymatic reaction. Comparing the CV results shown in figure 3a, the self-assembly of TMV1cys and the further conjugation of GOx with CL resulted in a corresponding decrease in charge transfer kinetics, showing an increasing separation between the redox peak potentials. The range of the peak separations and the comparable levels between the oxidation and reduction peak intensities imply that the charge transfer reaction is in a quasi-reversible regime, indicating that the resulting currents are predominately governed by the rate of electron transfer rather than being diffusion-limited (Nernstian) [5]. This is attributed to reduced ion accessibility to the electrode surface resulting in a decrease in the rate of electron transfers at the surface. However, unlike the previously reported cases [6], where electrodes functionalized with smaller organo-thiol molecules showed no electrochemical current response, the TMV1cys and the GOx bioconjugation steps still did not completely block the electrode surface. This is likely due to their relatively large 3-D structures which leaves “pin-holes” between the assembled TMV1cys particles or via the central channels of TMV1cys, allowing for electrochemical conversion of the enzymatic reactions. Based on the change in electrode kinetics with TMV1cys/CL/GOx conjugations, the experiments in the following section, consisting of characterizing

the electrochemical conversion of the enzyme-based catalytic reaction, were carried out at redox potentials around -0.3 V (cathodic reaction) and ~0.5 V (anodic reaction) vs. Ag/AgCl.

As shown in figure 3b, a $Fe(CN)_6^{3-/4-}$ redox couple was needed as an electron mediator to convey enzymatic reaction-generated electrons to the Au electrodes (mediated electron transfer, MET). This was necessary as the direct electron transfer (DET) mechanism was not present - no redox current peaks were observed in the CV curve in the presence of glucose without the mediator molecule. This is attributed to the use of planar Au as an electrode material which does not create a desirable arrangement of GOx on the conductive element - the cofactor/redox center, flavin adenine dinucleotide (FAD), of GOx should be directly accessible by the conductive medium with a stringent theoretical distance requirement (<1.5 nm) [7].

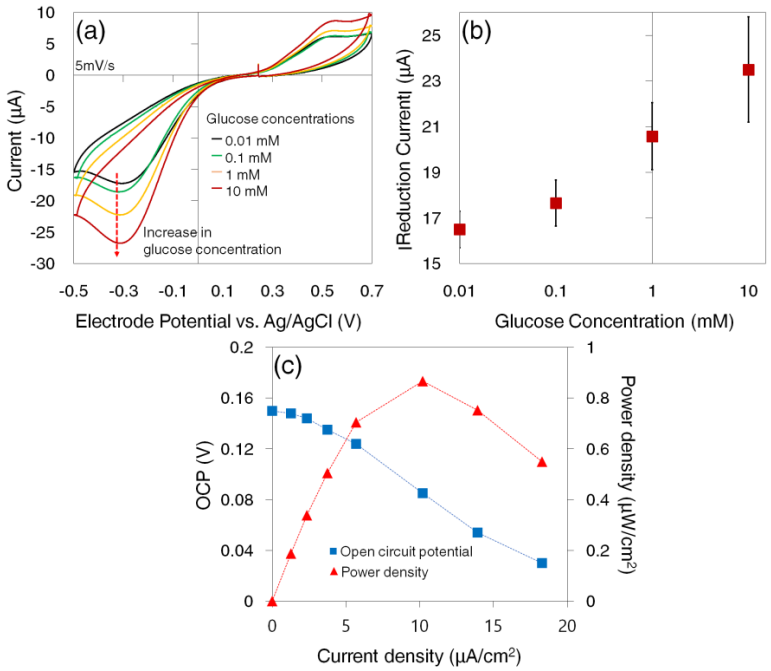

Figure 4. Electrochemical characterizations showing (a, b) increase in redox currents corresponding to glucose concentrations and (c) a polarization curve acquired from Au/TMV1cys/CL/GOx -SA (anode) – Pt (cathode) assembled EBC.

With the MET, a CV response corresponding to the glucose concentration was achieved (figure 4a) confirming successful electrochemical conversion of the enzymatic reaction. Figure 4b plots a calibration curve of the absolute reduction current corresponding to the glucose concentration levels (10 uM - 10 mM) demonstrating excellent performance of the Au/TMV1cys/CL/GOx-SA electrodes as glucose sensors. It should be noted that the reduction current was selected for determining the sensing capability as the oxidation currents showed rate-determined behavior with limited current levels compared to the reduction currents. Particularly, the results can be compared with a recent similar work by Bäcker et al. [8], where they demonstrated glucose sensing with post-immobilization of TMV1cys/CL/GOx on Pt electrodes. Comparing the current densities at the same glucose concentration, more than a 20-fold increase in current density (~3.4 $\mu A/mm^2$ vs. ~0.13 uA/mm^2 at 10 mM glucose) is achieved in this approach. The difference is most likely due to 1) the increased GOx immobilization density with high-density self-assembly of TMV1cys on Au surface and 2) the alternative/advanced generation of glucose sensing mechanism (2^{nd} generation of glucose sensors relying on MET) incorporated in this work, whereas the previous work relied on direct reduction of H_2O_2, produced from the enzymatic reaction on Pt electrodes (1^{st} generation of glucose sensors).

The bioenergy harvesting performance of the Au/TMV1cys/CL/GOx-SA (anode) - Pt (cathode) assembled EBC has been characterized as shown in figure 4c. Ferri/ferrocyanide (1 mM $Fe(CN)_6^{3-/4-}$)

and 10 mM glucose were included to facilitate the anodic electrode reactions, and the ambient dissolved oxygen was used as the fuel for the cathodic oxygen reduction reaction on Pt [9]. Considering the electrode reduction potential for O_2 on a Pt electrode (vs. Ag/AgCl) is ~0.61 V (neutral pH), the OCP of the assembled biofuel cell is anticipated to be within a ~ 0.1 – 0.15 V range considering that the oxidation reaction of the GOx electrode is occurring at around 0.5 V vs. Ag/AgCl (figure 4a). This was evident in the results shown in figure 4c: the OCP (with no resistive load connected) is measured to be ~ 149 mV and shows a typical response of decreasing OCP/load potential with increasing current density due to the limited charge flow kinetics in fuel cell systems [1]. Based on the polarization curve shown in figure 4c, the EBC generated a maximum power density of 0.86 $\mu W/cm^2$ at a current density of 10.2 $\mu A/cm^2$.

4. Summary

In this work, an on-chip bioconjugation method has been developed for GOx immobilization using TMV1cys as a robust high-density molecular assembly platform. The colorimetric assay using HRP and luminol confirmed the on-chip process was effective, showing higher enzymatic activity for the Au/TMV1cys/CL/GOx-SA compared to the positive controls. Particularly, compared to a recent work using a different immobilization method with the same bioconjugation chemistry, the developed method provides a more efficient biofabrication strategy by leveraging optimized sample volumes/concentrations and relying on the specific binding mechanisms between the biological template and the device surface. In addition, the electrochemical characterization results provide fundamental understanding behind the electrochemical conversion and charge transfer mechanisms. Combined with the initial EBC performance characterization results, these imply exciting potential for the development of high performance EBCs for next-generation energy solutions for advanced medical devices.

Acknowledgement

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