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# Polymer-Immobilized, Hybrid Multi-Catalyst Architecture for Enhanced Electrochemical Oxidation of Glycerol

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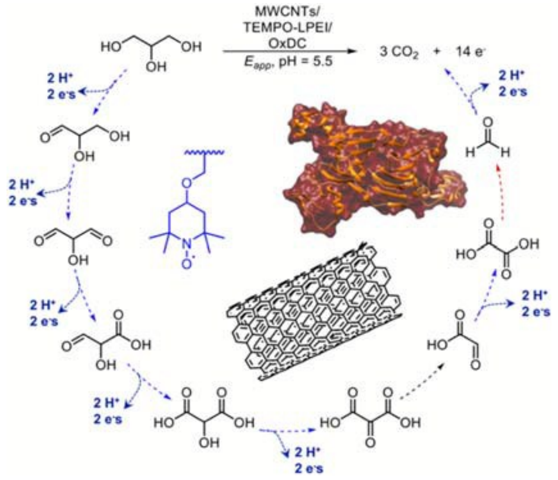
**The development of a hybrid, tri-catalytic architecture is demonstrated by immobilizing MWCNTs, TEMPO-modified linear poly(ethylenimine) and oxalate decarboxylase on an electrode to enable enhanced electrochemical oxidation of glycerol. This immobilized, hybrid catalytic motif results in a synergistic 3.3-fold enhancement of glycerol oxidation and collects up to 14 electrons per molecule of glycerol.**

Hybrid biofuel cells combine biological, small molecular, and/or metallic catalysts to electrochemically oxidize high-energy density fuel sources.[1-3] Complex biofuels constitute a unique and abundant subset of such molecules that can be electrocatalytically oxidized to generate electricity.[4, 5] By utilizing multiple catalytic motifs, it is possible to facilitate multiple oxidative steps for a single fuel molecule.[3, 6, 7] However, integration of additional catalytic modalities becomes increasingly complicated by the disparate reaction conditions required by each additional catalyst, as well as limitations arising from slow substrate diffusion between adjacent catalytic sites.[7] In addition, the combination of homogeneous and heterogeneous catalytic motifs often requires relatively high concentrations of the homogeneous component (i.e. high enzyme or small molecule catalyst concentration). Therefore, there remains a pressing need to design cost-effective molecular constructs that can efficiently integrate multiple catalytic motifs without a deleterious impact to the overall catalytic activity, while enabling deep electrochemical oxidation of fuels.

In this context, glycerol has been established as a promising fuel source due to its exceptionally high energy density[4, 8]; however, electrochemical conversion of glycerol to $CO_2$ presents a unique challenge due to the variety of reactions required, including primary and secondary alcohol oxidations to the resultant carbonyl compounds, aldehyde oxidation to a carboxylic acid and carbon-carbon bond cleavage processes. While the possible oxidative pathways from glycerol to tartrate, and subsequently to mesoxalate, are numerous and may be ambiguous, it is often the cleavage of $CO_2$ from mesoxalate and oxalate that is process-limiting in the oxidation cascade.[8-10] We have previously employed a hybrid catalytic approach that utilizes a simple organic catalyst, TEMPO, to electrochemically mediate alcohol and aldehyde oxidation in the first five steps of the glycerol oxidation pathway (Scheme 1).[11]



**Scheme. 1** Electrocatalytic oxidation cascade of glycerol by MWCNT/TEMPO-LPEI/OxDc multi-catalytic system. Oxidation reactions catalyzed by TEMPO-LPEI are indicated by blue arrows (steps 1 to 5, 7, 9), while decarboxylation reactions catalyzed by MWCNTs and OxDc are shown in black (step 6) and red (step 8) arrows, respectively.

This catalyst has been reported to electrochemically oxidize primary and secondary alcohols to aldehydes and ketones, respectively, as well as aldehydes to carboxylic acids under aqueous conditions.[12-19] However, nitroxyl radical catalysts are not known to oxidatively cleave $CO_2$. To cleave carbon dioxide from oxalate in the later steps of the cascade, we previously employed an enzyme, oxalate decarboxylase (OxDc), in creating a hybrid catalytic system to cleave $CO_2$ from oxalate in the later steps of the cascade.[20] Yet, even under optimum pH and temperature, the rate of $CO_2$ cleavage from mesoxalate by OxDc is slow when compared to TEMPO-mediated electrochemical oxidation of alcohols and aldehydes due to low promiscuity of the enzyme catalyst.[20] Furthermore, the high bulk

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† Electronic Supplementary Information (ESI) available: Experimental section, catalytic cyclic voltammograms, titration curves, and GC spectra as noted in the text. See DOI: 10.1039/x0xx00000x

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enzyme concentration required to compensate for catalytic deficiency of OxDc is both cost and space prohibitive on the electrode surface. We hypothesized that this problem may be addressed by co-immobilizing three promiscuous catalytic motifs, a small organic catalyst such as TEMPO, an enzymatic catalyst and catalytically active carbon nanotubes, to target process-limiting steps and selectively enhance the overall rate of glycerol oxidation.

Herein, we demonstrate the rational incorporation of three catalytic motifs onto a single electrode configuration to increase the overall oxidation rate of glycerol to $CO_2$. Specifically, we employed a recently developed TEMPO-modified linear poly(ethylenimine) (TEMPO-LPEI) as a molecular scaffold to simultaneously immobilize TEMPO, carboxylated multi-walled carbon nanotubes (COOH-MWCNTs) and OxDc directly onto an electrode surface to catalyze alcohol oxidation, cleavage of $CO_2$ from mesoxalate, and hydrolytic $CO_2$ cleavage of oxalate, respectively. Our strategy relies on the ability of the polymer hydrogel, TEMPO-LPEI, to effectively immobilize OxDc without substantial detriment to its catalytic activity, as well as the ability of catalytically active MWCNTs to facilitate the cleavage of carbon dioxide from mesoxalate. By confining them into a single electrode configuration using crosslinking chemistry as opposed to a typical solution-based technique, we eliminated the need for additional catalyst diffusion steps to the interrogating electrode. Consequently, this enables faster electron transfer rates between the substrates, catalysts and the electrode surface.

Prior to hybridizing with an enzyme, we first tested the electrocatalytic activity of immobilized TEMPO-LPEI against glycerol, glyceraldehyde and formate, which generated maximum current densities of 0.70 mA $cm^{-2}$, 0.16 mA $cm^{-2}$, and 2.86 mA $cm^{-2}$, respectively. This confirms previous findings that TEMPO-LPEI is capable of rapidly oxidizing several key intermediates of the glycerol oxidation cascade. Furthermore, TEMPO-$NH_2$-based systems have been shown to catalyze the first five oxidation reactions of the glycerol oxidation cascade and produce mesoxalate as the intermediate.[20, 21] However, not all TEMPO derivatives are able to catalyze the electrochemical oxidation of tartrate to mesoxalate, adding a further impediment to completing the cascade.[21] Thus, we initiated our study by examining the hybrid, multi-catalytic system for the electrochemical oxidation of tartrate. Our data demonstrates that immobilized MWCNT/TEMPO-LPEI/OxDc exhibits catalytic activity towards tartrate, as evidenced by the increased oxidative current (~3.89 mA $cm^{-2}$ at 0.70 V vs. SCE) in the presence of tartrate (Fig. S1, ESI†). When compared to relevant controls, catalytic oxidation of tartrate was not observed, as shown by the absence of an oxidative electrocatalytic wave for the immobilized MWCNTs, as well as lack of increase in the kinetic activity of OxDc in the presence of tartrate (Fig. S1, ESI†). This implies that tartrate oxidation is not contributing significantly to the process-limiting steps of the oxidation cascade and confirms the ability of the multi-catalyst system in mediating the first five oxidation steps of the glycerol oxidation cascade.

In fabricating the hybrid MWCNT/TEMPO-LPEI/enzyme modality, we first attempted to cross-link MWCNT/TEMPO-LPEI with oxalate oxidase (OxOx), an oxidoreductase enzyme that will catalyze the oxidation of oxalate. However, the enzyme had low specific activity[20, 22, 23] (data not shown) and notable limited overlap with the working pH range of TEMPO.[21, 23] In light of this, we explored the possibility of hybridizing and immobilizing a non-redox enzyme, oxalate decarboxylase (OxDc), with TEMPO-LPEI, as this enzyme has been demonstrated to exhibit higher substrate activity and catalytic response when challenged with mesoxalate and oxalate.[20] While OxDc may not be a conventional enzyme that is typically employed in bioelectrocatalysis, its utility was logical for our purpose. Specifically, we were enthusiastic about its high specific and catalytic activity,[20, 24-26] ease of expression and purification, and amenability to protein bioengineering.[20, 22, 24] Moreover, OxDc should catalyze the decarboxylation of oxalate via cleavage of the C–C bond, producing formic acid as an intermediate, which can efficiently be converted to $CO_2$ by TEMPO-LPEI, as reported previously.[20, 27]

We evaluated the catalytic ability of the hybrid MWCNT/TEMPO-LPEI/OxDc system to electro-oxidize mesoxalate, oxalate, and formate by carrying out constant potential amperometry with the potential held at 0.70 V (vs. SCE) for all systems. Fig. 1 shows comparative amperometric titration curves for the immobilized MWCNT/TEMPO-LPEI/OxDc systems when challenged with increasing concentrations of mesoxalate, oxalate, and formate. The titration curves reveal distinct differences in the electrocatalytic activities of the hybrid system towards intermediates involved in the rate-limiting steps of the glycerol oxidation cascade, generating maximum current densities of ~0.5 ± 0.0 mA $cm^{-2}$, ~2.0 ± 0.3 mA $cm^{-2}$, and ~2.7 ± 0.4 mA $cm^{-2}$ for mesoxalate, oxalate, and formate, respectively. Noticeably, the multi-catalytic system exhibits the highest catalytic current densities (~2.7 ± 0.4 mA $cm^{-2}$) in the presence of formate (within the concentration range of 0 – 60 mM) and may be attributed to the high activity of TEMPO-LPEI towards formate.[27]

The use of concentrations lower than 60 mM in the titration experiments is due to the insolubility of mesoxalate and oxalate at high concentrations (> 60 mM) even with constant stirring, as well as observed delamination of the films from the electrode surface at high substrate concentrations. We attribute the instability (i.e., film delamination) of the cross-linked films to the rapid production of bubbles at the electrode surface when the substrate concentration is high (indicative of $CO_2$ production), which significantly weakens the cross-linking of the polymer to the surface as the gas bubbles become entrapped in the hydrogel matrix especially during swelling. Of note, the TEMPO-LPEI hydrogel polymer is known to swell to facilitate rapid diffusion of the substrates, which is one of the reasons why this system was selected to act both as an oxidation catalyst and as an anchor onto an electrode surface.[27]

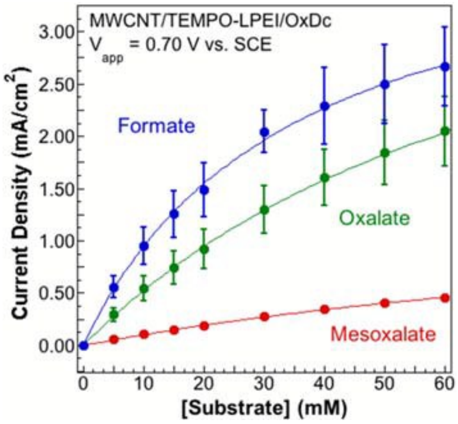

**Fig 1.** Catalytic oxidation of mesoxalate (*red curve*), oxalate (*green curve*), and formate (*blue curve*) by hybrid MWCNT/TEMPO-LPEI/OxDc immobilized on a carbon electrode surface. Amperometric titration curves obtained with $E_{app}$ = 0.70 V vs. SCE. Data points fitted into a Michaelis-Menten model.

To confirm if the catalytic current densities observed for the hybrid catalytic systems were in fact caused by the enzymatic activities

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towards mesoxalate, oxalate, and formate and not just due to the high reactivity of the TEMPO-LPEI catalyst, we carried out control experiments by immobilizing denatured OxDc to deactivate or lower the enzyme activity, and compared the resulting activities against the active OxDc. The oxidative current densities of both the active and denatured forms of OxDc did not show significant difference in catalytic activity towards mesoxalate (Fig. 2) indicating that the enzyme was not involved in the catalytic reaction of mesoxalate to glyoxylate. Since TEMPO-LPEI is not known to catalyze the cleavage of $CO_2$ from mesoxalate, this strongly suggests that MWCNTs were catalytically-active towards mesoxalate and catalyzed the decarboxylation of mesoxalate (Fig. 1, red curve and Fig. 2). Comparative cyclic voltammograms obtained for bare (Fig. S2, left, ESI†) and MWCNT-modified (Fig. S2, right, ESI†) electrodes also confirm this observed catalytic activity of MWCNTs towards mesoxalate, as evidenced by the occurrence of an oxidative wave starting at ~ 0.6 V vs. SCE.

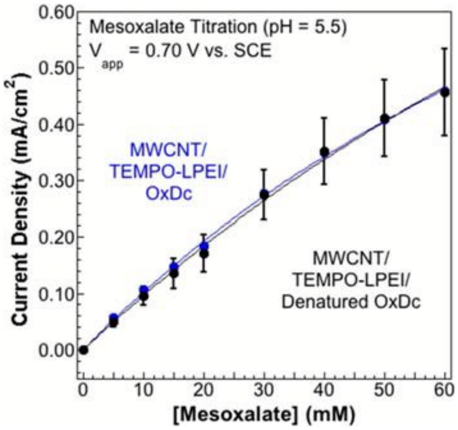

**Fig. 2.** Catalytic oxidation of mesoxalate by immobilized MWCNT/TEMPO-LPEI/OxDc. Titration curves obtained for the active (*blue curves*) and denatured (*black curves*) forms of OxDc in the presence of mesoxalate.

In contrast, the active form of OxDc (Fig. 3, blue curve) demonstrated higher activity towards oxalate than the denatured form (Fig. 3, black curve), which implies that the oxidative current densities obtained were caused by enzyme activity and not just by the catalytic activity of the TEMPO-LPEI polymer.

We also confirmed this result by performing an activity kinetic assay for both the solution-based (Fig. S3, black curve, ESI†) and immobilized OxDc (Fig. S3, blue curve, ESI†) against increasing concentrations of oxalate. Titration curves obtained for the solution-based OxDc and immobilized MWCNT/TEMPO-LPEI/OxDc generated $V_{max}$ values of 0.366 ± 0.008 μmol min$^{-1}$ and 0.066 ± 0.002 μmol min$^{-1}$, respectively (Fig. S3, ESI†). The decrease in enzyme activity observed for the cross-linked films can be attributed to diffusion limitations caused by immobilization.[27] Nevertheless, the immobilized hybrid system still exhibited significant activity towards oxalate (0.066 ± 0.002 μmol min$^{-1}$), thereby supporting the previous findings, as well as verifying that OxDc is still active even when immobilized on an electrode surface. Finally, the current densities were generally higher for both the active (Fig. S2, blue curve, ESI†) and denatured OxDc (Fig. S2, black curve, ESI†) when formate was the substrate, indicating that the TEMPO-LPEI polymer effectively catalyzes the oxidation of formate, either in the presence or absence of OxDc, consistent with the literature.[20, 27]

These results show that the catalytic current densities observed when OxDc is deactivated (denatured) are due to the TEMPO-LPEI and MWCNTs acting on their corresponding substrates, but when OxDc is active and carries out the decarboxylation of oxalate, a synergistic enhancement in the electrocatalytic oxidation of glycerol is enabled. As these results provide evidence of the ability of the hybrid system to catalytically transform mesoxalate, oxalate and formate – the last three intermediates contributing to the rate-limiting steps of the cascade (Fig. 1), we sought to demonstrate the potential of this hybrid multi-catalytic system in enhancing the rate of oxidation of glycerol to produce $CO_2$, and verify that the oxidation cascade for glycerol can occur at ambient conditions. Therefore, we examined the ability of the hybrid system to electrochemically oxidize glycerol to $CO_2$, on the basis that the MWCNTs and OxDc will perform cleavage of the C–C bond of mesoxalate and oxalate, respectively, and facilitate faster rate of electrochemical oxidation of glycerol.

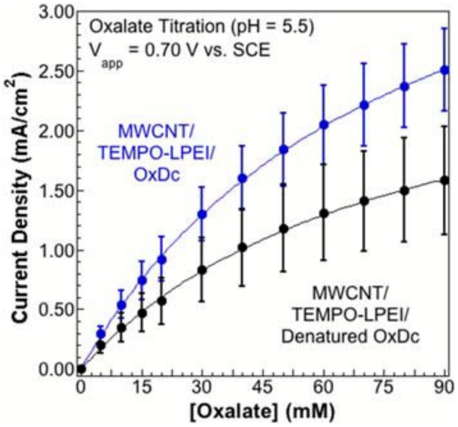

**Fig. 3.** Catalytic oxidation of oxalate by immobilized MWCNT/TEMPO-LPEI/OxDc. Titration curves obtained for the active (*blue curves*) and denatured (*black curves*) forms of OxDc in the presence of oxalate.

Employing similar experimental conditions as discussed above, we performed a series of cyclic voltammetric experiments in the presence of increasing concentrations of glycerol using the MWCNT/TEMPO-LPEI/OxDc hybrid system (Fig. 4, blue curve) and MWCNT/TEMPO-LPEI as a control ((Fig. 4, black curve) and catalytic current densities at 0.70 V (vs. SCE) were plotted against the concentration of glycerol (Fig. 4). It is evident from Fig. 4 that a significant 3.3-fold enhancement in the peak oxidative current density is obtained for the hybrid MWCNT/TEMPO-LPEI/OxDc system in the presence of 1 M glycerol, generating a current density of ~1.3 ± 0.2 mA cm$^{-2}$, as opposed to a current density of ~0.4 ± 0.1 mA cm$^{-2}$ generated by the MWCNT/TEMPO-LPEI system (control). This data strongly suggests that the immobilized, hybrid MWCNT/TEMPO-LPEI/OxDc multi-catalytic system enables enhanced electrochemical oxidation of glycerol to $CO_2$, as a result of the combined catalytic activities of the enzyme, MWCNTs, and the organic polymer towards the substrates involved in the process-limiting steps of the glycerol oxidation cascade. We also confirmed the production of $CO_2$ by performing a 24-hour bulk electrolysis on the immobilized MWCNT/TEMPO-LPEI/OxDc in the presence of 50 mM glycerol and used gas chromatography to detect the formation of $CO_2$ in the headspace (Fig. S4, blue curve, ESI†).

Consistent with our proposed scheme (Scheme 1), we observed that enhanced electrocatalytic activity of the hybrid system towards glycerol is a consequence of the ability of the TEMPO-LPEI polymer

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to effectively catalyze the first five oxidation steps of glycerol to mesoxalate (collecting up to 10 e$^-$ from these steps), where mesoxalate is then acted upon by MWCNTs to undergo decarboxylation involving cleavage of a carbon-carbon (C–C) bond to form glyoxylate. The glyoxylate intermediate is then oxidized by TEMPO-LPEI to produce oxalate, generating an additional 2 e$^-$, followed by OxDc-catalyzed decarboxylation of oxalate to generate formate and $CO_2$ products. This last step permits the collection of an additional 2 e$^-$, since formate is easily oxidized to $CO_2$ by the TEMPO-LPEI polymer with a fast rate of oxidation while producing high current densities. This completes the glycerol oxidation cascade, with the hybrid MWCNT/TEMPO-LPEI/OxDc catalytic system able to harvest up to 14 electrons per molecule of glycerol. This presents promise as a bioanode material capable of exhibiting high rates of oxidation, for applications useful in the development of enzymatic biofuel cells.

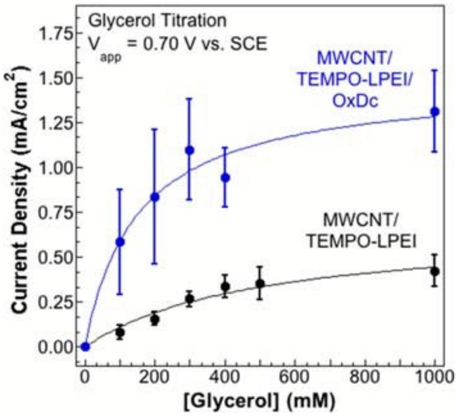

**Fig 4.** Glycerol-induced changes in the catalytic oxidation current by immobilized MWCNT/TEMPO-LPEI (*black curve*) and MWCNT/TEMPO-LPEI/OxDc (*blue curve*). The hybrid multi-catalytic system generates current densities of 0.4 ± 0.1 mA cm$^{-2}$ and 1.3 ± 0.2 mA cm$^{-2}$ for the MWCNT/TEMPO-LPEI and MWCNT/TEMPO-LPEI/OxDc hybrid systems, respectively. Data points fitted into a Michaelis-Menten model.

In summary, we demonstrate the abilities of three catalytic motifs (MWCNT, TEMPO-LPEI, and OxDc) to synergistically catalyze the oxidation of substrates involved in the process-limiting steps of the glycerol oxidation cascade. This enables the deep electro-oxidation of glycerol at an electrode surface, as well as the collection of up to 14 electrons per molecule of the fuel. Furthermore, immobilization of the hybrid catalytic motifs enables the construction of a practical catalytic architecture that minimizes the catalyst loading required for rapid and complete oxidation of glycerol to $CO_2$. The hybrid system also generates high catalytic current densities when compared to a system without an enzyme incorporated. This opens up a number of exciting opportuntites for the fabrication of new hybrid bioanode architectures capable of high rates of electrochemical oxidation.

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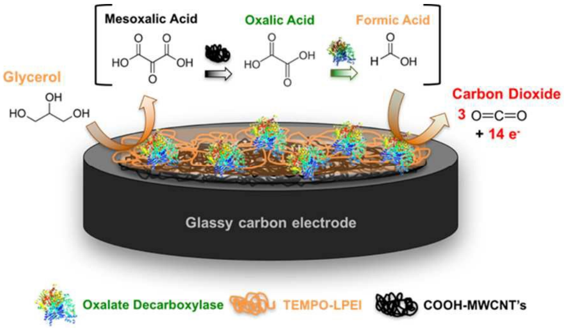

Polymer-immobilized MWCNT/TEMPO-LPEI/OxDc hybrid tri-catalytic motif enables a synergistic enhancement in the complete oxidation of glycerol.