Bioelectronic Devices

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A Self-Powered "Sense-Act-Treat" System that is Based on a Biofuel Cell and Controlled by Boolean Logic**

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Enzymatic biofuel cells (BFCs) have attracted considerable recent interest owing to their ability to provide sustainable energy from renewable fuel sources under mild conditions.[1] The ability to engineer these devices to process various "renewable" biochemical species holds considerable promise for the utilization of BFCs as implantable power sources for biomedical devices.[1,2] As well as major research efforts that are focused on extended operational stability, improved power efficiency, and device miniaturization,[1–3] recent activity has been devoted to the development of BFC-based logic biosensors with potential applications in the self-powered, biocomputing diagnostics domain.[4] In line with the principles of Boolean logic, such self-regulating, self-powered, implantable devices could be able to extract and process analytes that reside in complex media.[5]

Herein, we describe a self-powered, logic-controlled, integrated "sense-act-treat" system that is based on a BFC. Controlled drug-release technologies leverage the ability to deliver on-demand targeted therapies and thus have advantages over conventional methods, which include more rapid intervention capabilities and more effective doses.[6] The BFC-based system that has been developed in this study offers simultaneous, intelligent biocomputing diagnostic operations and controlled drug-release functionality without the need for external power sources. The use of a biocomputing-based detection method to trigger the release of a drug through the logic-based control of the BFC's power output has not been reported to date. In stark contrast to conventional biosensors or controlled-release systems, the BFC-based biocomputing system is able to autonomously correlate the relationship between different biomedically relevant markers according to "programmed" Boolean logic operations, and to regulate the release of a drug to counteract the onset of abnormal physiological states. The simultaneous, self-powered diagnostic operation and the controlled-release functionality are accomplished through a logic-induced change in the electrocatalytic activity at the anode, which triggers the release of a model therapeutic agent from the drug-loaded cathode. This logic-activated drug-release system could serve as the core component of an autonomous system for medical diagnoses and intelligent drug delivery that circumvents the need for any external power sources, control electronics, or microelectromechanical actuators.

Figure 1 shows the configuration of the "sense-act-treat" system. The system consists of an enzyme-based logic-controlled anode and a drug-containing cathode that is functionalized with a conducting polymer (CP). Under "normal" scenarios, the BFC is OFF and the drug remains embedded (doped) within the CP-modified cathode. Conversely, in the "abnormal" state, the Boolean logic anode will switch the BFC to the ON state, which results in the release (undoping) of the therapeutic agent from the cathode, in

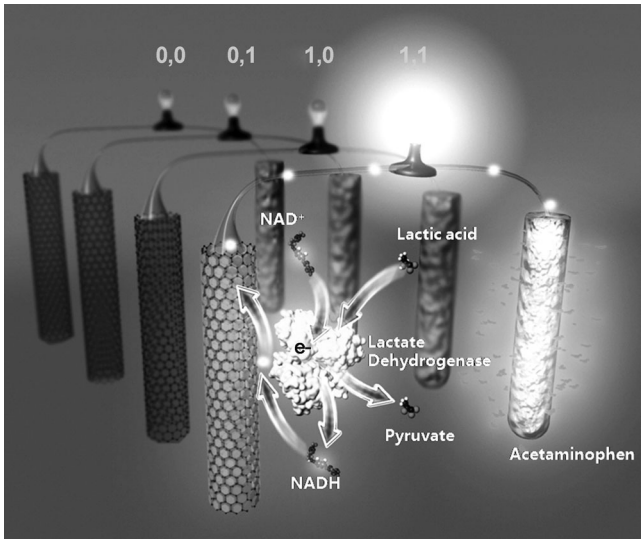

Figure 1. Illustration of the self-powered, biocomputing, logic-controlled "sense-act-treat" system based on a BFC. LAC and LDH are used as diagnostic biomarker inputs for the AND logic operation in connection with the ABT model injury. APAP is used as the model drug. In the presence of both inputs ((1,1) state), the BFC is switched ON, logically activating the release of the drug.

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conjunction with a substantial increase in the power output (as well as the corresponding open-circuit potential). In this manner, the digitally processed information yields a final ON/OFF output, thereby enabling the direct coupling of the logic detection method with the drug-release contingent and completes the integrated "sense-act-treat" system.

To demonstrate the concept of self-powered, logic-activated therapeutic intervention, we relied on an enzyme-based, logic-controlled anode (glassy carbon electrode modified with carbon nanotubes/Meldola's blue (CNT–MDB/GC electrode)) and a drug-loaded, CP-modified cathode ((poly(3,4-ethylenedioxythiophene)–acetaminophen (PEDOT–APAP) functionalized gold electrode) operated in a potassium phosphate buffer solution (PBS; 0.1 M, pH 7.4) that contained nicotinamide adenine dinucleotide (NAD^+) as a cofactor (20 mM, see the Supporting Information for details). Abdominal trauma (ABT) was selected as the model injury, along with the corresponding lactic acid (LAC) and lactate dehydrogenase (LDH) biomarker inputs, as well as the AND logic implementation.[7] The AND logic is represented by the situation where the gate output is activated only when both biomarker inputs are present.[8] The absence of LAC (input A) and LDH (input B) is considered as the logic input 0, whereas LAC (20 mM) and LDH (10 U mL^{-1}) are defined as logic input 1. Thus, these inputs provide a logic-based diagnosis of ABT. APAP, which is frequently used to control pain in abdominal injury, was selected as the model drug.[9]

Figure 2A shows the temporal response in the open-circuit potential at the anode after the application of different combinations of the biomarker inputs. In the absence of either or both of the inputs ((LAC, LDH) = (0,0), (0,1) and (1,0)), that is, "normal" states, the enzymatic reaction did not ensue and NADH was not produced. As a result, no appreciable alteration in the NADH oxidation open-circuit potential was detected, that is, the BFC remained in an OFF state. However, in "abnormal" scenarios in which LAC and LDH were both present (input (1,1)), the LDH-catalyzed enzymatic reaction of NAD^+ and LAC proceeded, which resulted in the formation of NADH and pyruvate; accordingly, the open-circuit potential for the oxidation of NADH substantially decreased from 0.18 V to −0.10 V (input (1,1) in Figure 2, ON state). As illustrated below, the large swing in the open-circuit potential leads to the concomitant release of the drug from the cathode upon assembling the complete BFC. The corresponding bar diagrams (Figure 2B) illustrate that only the (1,1) input combination resulted in the ON output state upon the application of the four logic combinations. Therefore, the features of the anode correspond to the equivalent functional operation of an AND logic gate that is able to perform the Boolean logic operation of A·B.[8] Owing to the high dynamic range that is associated with the AND logic gate, such an operation thus facilitates the straightforward discrimination between the OFF and ON output signals, as the open-circuit potential threshold of 0.15 V provides a clear threshold to separate "normal" and "abnormal" conditions.

Subsequently, the self-powered, logic-based diagnosis was paired with a logic-activated drug-release system that is

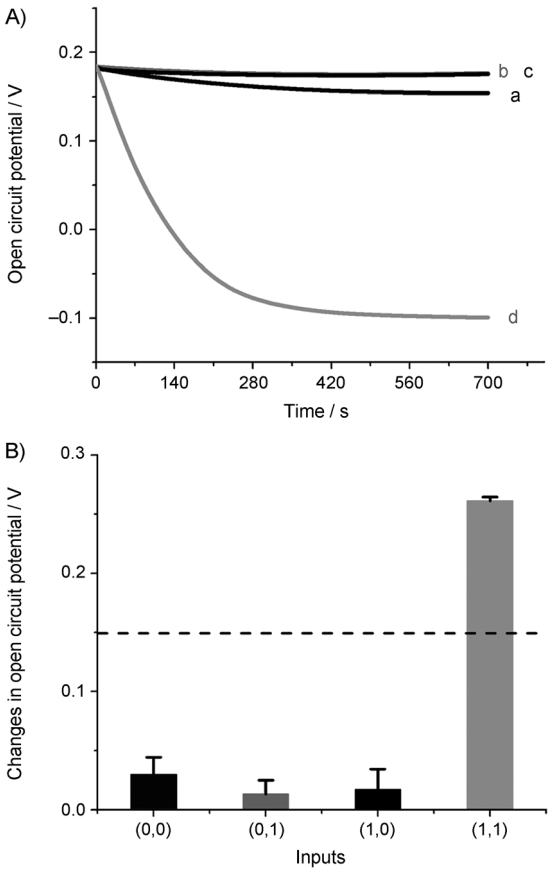

Figure 2. A) Temporal response of the open-circuit potential at the anode after the application of different input signals: a = (0,0), b = (0,1), c = (1,0), and d = (1,1). B) Bar diagrams showing the changes in open-circuit potentials at the anode for the different combinations of the input signals, derived from Figure 2A. The dashed line is at the threshold (0.15 V). Input A, LAC (20 mM); input B, LDH (10 U mL^{-1}). The respective combinations of the inputs LAC and LDH, as well as the NAD^+ cofactor (20 mM), were mixed and the reaction used for 30 min before measurements were recorded.

comprised of a cathode modified with PEDOT-APAP. CPs have been widely used in controlled drug-release devices because of their unique redox, doping/undoping, and meso/nanoporous properties.[6] The release of drug dopants that are entrapped within polymer hosts has been widely reported; the process is instigated by the application of a negative potential that serves to reduce, and thereby undope, the CP matrix host.[6] Data from preliminary control experiments suggested that only the application of a suitable redox potential of 0.0 V caused the PEDOT-APAP electrode to release the model drug (Figure S3 in the Supporting Information). Furthermore, the open-circuit potential at the PEDOT-APAP cathode, which corresponds to the reduction of PEDOT (for all input combinations) was higher than that at the anode (Figure S1 in the Supporting Information). Different combinations of the input signals, (0,0), (0,1), (1,0), and (1,1), did not alter the open-circuit potential of the cathode (Figure S1 in the Supporting Information). Thus, the PEDOT-APAP-modified electrode is suitable to serve as the cathode within the self-powered, controlled-release BFC.

Accordingly, the complete, compartment-free BFC was assembled from the logic-controlled anode and the drug-doped cathode. The corresponding power curves were obtained for the different combinations of the inputs (Figure 3A). Under the "abnormal" scenario when LAC and LDH were both present (input (1,1)), the BFC switched ON and gave a maximum power output of approximately

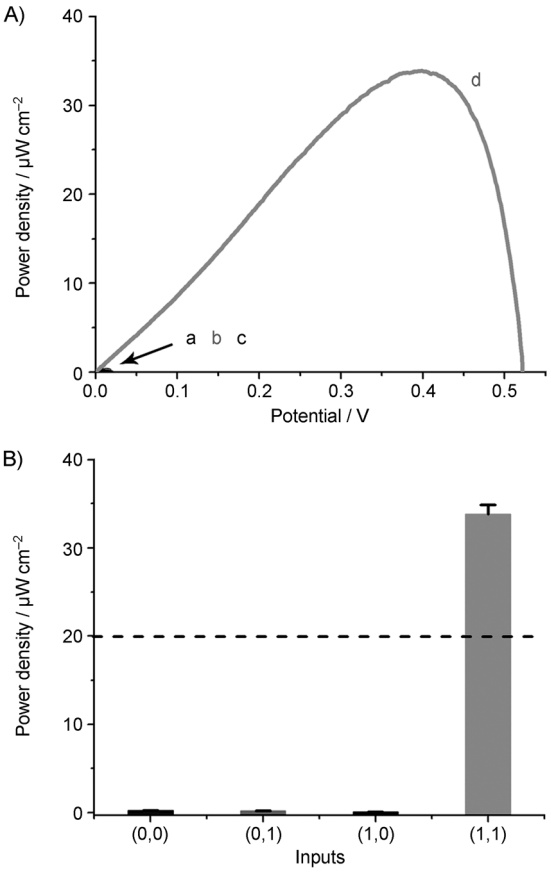

Figure 3. A) Dependence of the power density on the BFC voltage for different combinations of input signals a = (0,0), b = (0,1), c = (1,0), and d = (1,1). B) Bar diagrams showing the power density of the BFC for the different combinations of input signals, derived from Figure 3A. The dashed line is at the threshold (20 μW cm^{-2}). Input concentrations and reaction time are as in Figure 2.

33.8 μW cm^{-2} (at approximately 0.40 V, output = 1, line d, Figure 3). In contrast, when the (0,0), (0,1), and (1,0) input combinations were applied, that is, a "normal" state, only negligible alterations in the NADH oxidation open-circuit potential occurred, thereby switching the BFC OFF (output = 0, lines a, b, and c, Figure 3). Figure 3B shows the corresponding bar diagrams for the power output for the same four input combinations. The selected threshold value of 20 μW cm^{-2} conveniently separates the OFF and ON logic states of the output power signal, whereby only the presentation of the input combination (1,1) switches the output of the system to the ON state.

Consequently, the amount of the drug released by the complete BFC in response to different input combinations was examined by voltammetric measurements of APAP. Figure 4 shows the comparison of the current response that was obtained from differential pulse voltammograms (DPVs) at a CNT/GC electrode for the released APAP for the four

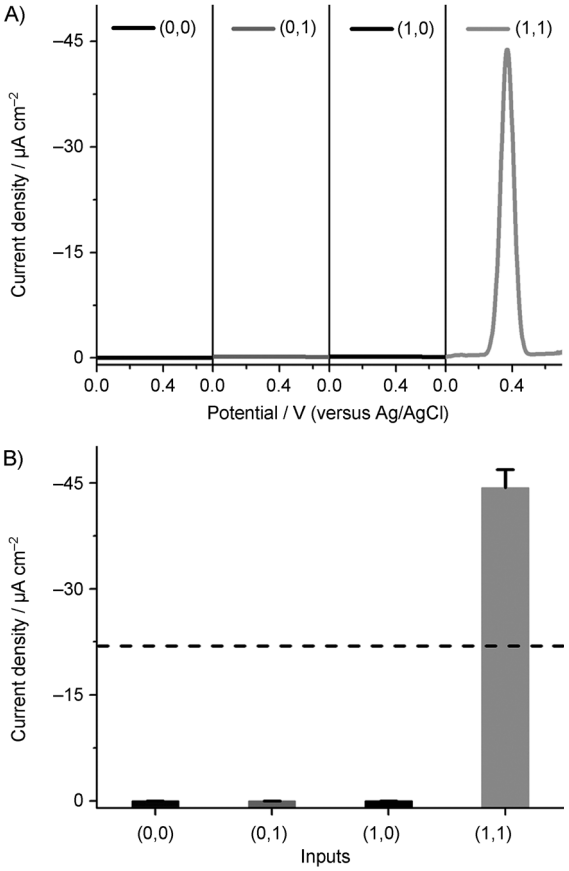

Figure 4. Logic-activated drug release. A) Differential pulse voltammograms at the CNT/GC electrode for the APAP released from the "sense-act-treat" system by using different combinations of the input signals (0,0), (0,1), (1,0), and (1,1). Initial potential: 0.7 V; final potential: 0 V; scan rate: 0.1 V s^{-1}. B) Bar diagrams showing the APAP current density response for the different combinations of the input signals, derived from Figure 4A. The dashed line is at the threshold (−22 μA cm^{-2}). Input A, LAC (20 mM); input B, LDH (10 U mL^{-1} Input reaction time as in Figure 2. Data were taken after 60 min of unperturbed BFC operation.

combinations of the input biomarkers. Only the (1,1) "abnormal" state resulted in an appreciable current response (Figure 4), hence, these results demonstrate that the sense-act-treat system releases significant quantities of APAP to counteract the onset of the abnormal state (Figure S4 in the Supporting Information). The substantial catalytic reactions at the anode are associated with the (1,1) state and dramatically increase the open-circuit potential, thus, initiating the undoping and concomitant release of the drug from the cathode. In contrast, no APAP signals were detected in the presence of the (0,0), (0,1), and (1,0) input combinations. No detectable undoping of the CP-modified cathode occurred in this "normal" (OFF) state, which reflects the negligible open-circuit potentials and power output (Figure 3). Overall,

the data presented in Figure 4 clearly illustrate that drug release was triggered to counteract the onset of the "abnormal" physiological states. Furthermore, the data from control experiments over a prolonged period of 120 min (after the 30 min input reaction between LAC, LDH, and NAD^+) indicated that no detectable DPV signals were generated by APAP (that is, drug release) upon the application of the inputs (0,0), (0,1), (1,0) or when the circuit was broken (Figure S4 in the Supporting Information). The results of the control experiments also suggested that drug release from the system was terminated when the targets were absent (Figures S4G and S4H in the Supporting Information).

We examined the influence of potential electroactive interferences upon the controlled drug-release operation. No apparent APAP release was detected in the presence of physiological levels of uric acid (UA), dopamine (DA), and epinephrine (EP). However, very minor release of APAP, which corresponds to approximately 6% and approximately 3% of the (1,1) level, was detected in the presence of physiological levels ascorbic acid (AA) and cysteine (CySH), respectively (Figure S5 in the Supporting Information). Practical biomedical applications would require proper attention to these small potential interferences, possibly by the incorporation of appropriate permselective coatings.[10]

In conclusion, we have demonstrated a biocomputing, logic-based detection method with a controlled-release drug-delivery actuator that is based on a closed-loop, self-powered BFC system. By harnessing built-in Boolean AND logic at the anode with physiologically relevant biomarker inputs, the proof of concept "sense-act-treat" system instigates a therapeutic intervention upon the detection of "abnormal" conditions through logic-based control of the BFC's power output. The concept of logic-activated therapeutic intervention could serve as the core component of an autonomous medical diagnostic and intelligent drug-delivery system that circumvents the need for external power sources, control electronics, or microelectromechanical actuators. The concept of using logic-activated delivery of a therapeutic intervention to counteract an abnormal state could thus be adapted to a plethora of diverse healthcare applications, and may lead to major improvements in patient care. However, significant progress is necessary before practical biomedical applications of this concept can be implemented, which include advances in the diagnostic capabilities of the logic gates, minimization of potential interferences, and attention to biofouling effects. Moreover, in order to extend the concept to practical in vivo applications, future devices will require further improvement, such as the covalent immobilization of the NAD^+ cofactor and optimization of the BFC parameters, to meet relevant "sense-act-treat" situations. Furthermore, the implementation of biochemical filters[11] is expected to improve the ON/OFF dynamic range and response time of the device and thus enable truly binary release of the model drug under diverse pathophysiological scenarios.

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