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Enzyme logic gates for the digital analysis of physiological level upon injury

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ABSTRACT

A biocomputing system composed of a combination of AND/IDENTITY logic gates based on the concerted operation of three enzymes: lactate oxidase, horseradish peroxidase and glucose dehydrogenase was designed to process biochemical information related to pathophysiological conditions originating from various injuries. Three biochemical markers: lactate, norepinephrine and glucose were applied as input signals to activate the enzyme logic system. Physiologically normal concentrations of the markers were selected as logic 0 values of the input signals, while their abnormally increased concentrations, indicative of various injury conditions were defined as logic 1 input. Biochemical processing of different patterns of the biomarkers resulted in the formation of norepi-quinone and NADH defined as the output signals. Optical and electrochemical means were used to follow the formation of the output signals for eight different combinations of three input signals. The enzymatically processed biochemical information presented in the form of a logic truth table allowed distinguishing the difference between normal physiological conditions, pathophysiological conditions corresponding to traumatic brain injury and hemorrhagic shock, and abnormal situations (not corresponding to injury). The developed system represents a biocomputing logic system applied for the analysis of biomedical conditions related to various injuries. We anticipate that such biochemical logic gates will facilitate decision-making in connection to an integrated therapeutic feedback-loop system and hence will revolutionize the monitoring and treatment of injured civilians and soldiers.

1. Introduction

The majority of battlefield, criminal or traffic accident deaths occur within the first 30 min after injury. Accordingly, rapid evaluation of physiological conditions and immediate treatment of an injured person are extremely important for enhancing the survival rate (Baker et al., 1992). Medical intervention without obtaining reliable information about the exact nature of the injury might be meaningless or even dangerous since various injury scenarios (brain injury, trauma, shock, fatigue, etc.) require different therapeutic treatments (Zasler et al., 2006; Feliciano et al., 2007; Altura et al., 1983). However, in many cases (particularly for injured soldiers in a battlefield) rapid evacuation to a hospital is impossible and the medical diagnosis is too late. Thus, high fidelity real-time diagnostics is of critical importance for reliable decision-making and optimal treatment immediately after the injury has occurred. Eventually, such reliable and rapid analysis of the type of injury could be coupled to an automatic drug-delivering feedback-loop, leading to a timely therapeutic intervention and hence to decreased mortality and severity of the post-traumatic conditions.

Application of standard medical analyses to an injured person requires hospital conditions and cannot be performed without sophisticated equipment and highly trained personnel. Evaluation of the injury conditions directly on an "on-the-spot" basis should involve novel approaches based on modern advances in the area of biosensors (Curreli et al., 2008; Mascini and Tombelli, 2008; Wang, 2006). However, a common biosensor device, selective to one specific target analyte, cannot provide sufficient information to make a medical diagnostics regarding the kind of injury and the required treatment. This conclusion should be based on the simultaneous analysis and comparison of several physiological markers that are known to vary for different types of injury. Following the traditional biosensor approach, several sensing devices should be applied simultaneously, all of them will be equipped with proper electrical and computer interfaces and energy sources, which will process the obtained information and make the final decision. The area of biotechnology involving the sensing and transduction of signals, for instance, for drug delivery in implantable devices, has been rapidly evolving. In this approach, each step increases in complexity of the "decision-making" algorithms involved, and the output

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of this system must interface with the external electronic devices required for further information/signal processing. Some engineering progress has been achieved in this direction through the use of biosensor arrays in restricted and safety-critical environments (Astaras et al., 2008). However, this approach is not practical in remote locations (e.g. for soldiers in a battlefield) and it has little advantages over regular diagnostics in a hospital. Thus, the formulated aim requires a novel unconventional approach to the decision-making (sense-and-treat) biosensor.

The recently pioneered concept of biochemical information processing (biocomputing) allows for the analysis of various chemical signals, their comparison and processing according to a special chemical built-in "program" using only chemical means without the involvement of electronic computers (Shao et al., 2002). Biochemical processing of information with biomolecules and biological components by realizing and networking logic "gates" that mimic Boolean digital logic offers great promise for clinical diagnostics. Indeed, biomolecules are selective and usable in complex biochemical environments. Such systems are also expected to be appropriate for interfacing of the biocomputing "devices" with processes in living organisms, and hence can be useful for future practical biomedical applications. Integration of biocomputing elements with sensing processes would allow multi-signal analysis followed by chemical processing of the data, giving the final answer regarding the physiological conditions of an injured person in a digital ("YES" or "NO") form. In the next development step, the biocomputing system – analyzing the biochemical information and making decision – could be connected to a chemical actuator releasing an appropriate drug to treat an injured person. Biocomputing elements of even moderate complexity will allow an effective interface between complex physiological systems and nano-structured signal-responsive materials and/or electronic systems. Thus, integration of the biocomputing concepts into sensing technology will contribute to a new emerging technological paradigm: networking of information processing stages that involve only biochemical processes, aiming at eliminating the "wires" and "batteries" and reducing the overall need for electrical power supplies at the stages of information processing, carried out on-site in implantable devices.

Biocomputing (Shao et al., 2002) being a sub-area of unconventional chemical computing (De Silva and Uchiyama, 2007; Credi, 2007) ranges from application of biomolecules (proteins, Tomizaki and Mihara, 2007; Unger and Moult, 2006; enzymes, Sivan et al., 2003; Strack et al., 2008a; Baron et al., 2006; DNA, Stojanovic et al., 2005) to the use of whole biological cells (Simpson et al., 2001) for processing biochemical signals in a digital form according to Boolean logic operations. Recent extensive research in the area of the enzyme-based logic systems allowed the formulation of different Boolean logic gates (Strack et al., 2008a; Baron et al., 2006). Further scaling up the complexity of the enzyme logic systems allowed for their concatenation in sophisticated logic networks processing various patterns of different chemical signals and generating final output signals with the encoded information dependent on the entire set of input signals (Strack et al., 2008b). Experimental evaluation and theoretical analysis of enzyme logic gates predicted that an acceptable noise level and error-free information processing could be achieved upon concatenation of at least 10 logic gates, processing many different combinations of input signals (Privman et al., 2008). The enzyme information processing systems were integrated with various electrochemical analyzing interfaces to transduce the resulting output signal from a chemical to electrical form (Zhou et al., 2009). Enzyme biocomputing systems were also shown to operate as logic switches for complex bioelectronic devices (Amir et al., 2009) and nano-structured signal-responsive materials performing various chemical actuation functions (Tokarev et al., 2009). Such integrated multi-functional systems composed of the biochemical information processing units, electronic transducers and chemical actuators would allow fast autonomous and reliable analysis of pathophysiological changes originating from an injury, identification of the type of injury, process the medical state in real time and performing proper drug-delivery intervention when physiologically appropriate. Application of enzyme logic systems coupled with signal-responsive materials and electronic transducers would thus allow for the analysis, decision-making and therapeutic intervention performed by pure chemical means without sophisticated electronic equipment, and hence could lead to improved survival rate among injured civilians and soldiers.

The present paper describes a novel approach to the biochemical analysis and data processing based on biocomputing with enzyme-based logic gates that eventually could be used for real-time field monitoring of injured people. Specifically, biocomputing systems analyzing biochemical markers, characteristic of different pathophysiological conditions, have been developed here to illustrate the potential biomedical application of biocomputing. The paper represents the advanced step of our recent research (Pita et al., 2009) with significant improvement of the signal processing applying a new set of logic operations which allow better discrimination of different pathophysiological conditions. Our data illustrate the ability to generate distinct patterns of output signals from different combinations of biochemical input signals, characteristic of various normal and abnormal physiological conditions. We anticipate that such biochemical logic gates will create high fidelity diagnosis for a greatly improved decision-making, leading to an optimal timely therapeutic intervention and to improved survival of injured soldiers and civilians. Keeping in mind this ultimate goal and aiming at minimally invasive enzyme logic gate based sensing device, we stepped forward from optical analysis of the enzyme logic operations to electrochemical transduction of the output signals. Both signal transduction methods (optical and electrochemical) were employed and compared, demonstrating good consistency of the results.

2. Experimental

2.1. Chemicals and reagents

Lactate oxidase (LOx) (E.C. 1.1.3.2) was purchased from Genzyme Corp. and was purified as follows. About 100 μL of LOx (0.5 units μL^{-1}) in a 50 mM phosphate buffer solution (pH 7.4) was taken in 100 kDa Centrisart ultra-filtration tube and centrifuged at 7000 rpm for 15 min at 4 °C. The sediment was washed with 80 μL of phosphate buffer and centrifuged. The process was repeated five times. All other chemicals and enzymes were purchased from Sigma–Aldrich and used as supplied without any further purification: β-nicotinamide adenine dinucleotide (NAD^+), D-(+)-glucose, (L)-norepinephrine (NE), L-(+)-lactic acid. Other enzymes used were horseradish peroxidase (HRP) type VI (E.C. 1.11.1.7) and glucose dehydrogenase (GDH) from *Pseudomonas* sp. (E.C. 1.1.1.47). Ultrapure water (18.2 MΩ cm) from NANOpure Diamond (Barnstead) source was used in all of the experiments.

2.2. Instruments

The optical measurements were performed using a Shimadzu UV-2450PC spectrophotometer. The electrochemical measurements were performed using a CH Instrument Model CHI630C with an electrochemical cell consisting of a glassy carbon working electrode, Ag/AgCl (3 M NaCl) reference and a platinum wire counter electrode.