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Review

Implantable enzyme amperometric biosensors

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ABSTRACT

The implantable enzyme amperometric biosensor continues as the dominant *in vivo* format for the detection, monitoring and reporting of biochemical analytes related to a wide range of pathologies. Widely used in animal studies, there is increasing emphasis on their use in diabetes care and management, the management of trauma-associated hemorrhage and in critical care monitoring by intensivists in the ICU. These frontier opportunities demand continuous indwelling performance for up to several years, well in excess of the currently approved seven days. This review outlines the many challenges to successful deployment of chronically implantable amperometric enzyme biosensors and emphasizes the emerging technological approaches in their continued development. The foreign body response plays a prominent role in implantable biotransducer failure. Topics considering the approaches to mitigate the inflammatory response, use of biomimetic chemistries, nanostructured topographies, drug eluting constructs, and tissue-to-device interface modulus matching are reviewed. Similarly, factors that influence biotransducer performance such as enzyme stability, substrate interference, mediator selection and calibration are reviewed. For the biosensor system, the opportunities and challenges of integration, guided by footprint requirements, the limitations of mixed signal electronics, and power requirements, has produced three systems approaches. The potential is great. However, integration along the multiple length scales needed to address fundamental issues and integration across the diverse disciplines needed to achieve success of these highly integrated systems, continues to be a challenge in the development and deployment of implantable amperometric enzyme biosensor systems.

Contents

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1. Introduction

An implantable enzyme amperometric biosensor is a bioanalytical technology that is intended to measure and often remotely transmit a record of specific molecular-level of a biological analyte within the human body (Vaddiraju et al., 2010). The basic function is to indwell a tissue and to detect, measure and record the levels of a molecule of interest within those tissues. Being amperometric, the biotransducer generally comprises two or three electrodes: a working electrode rendered specific to the analyte of interest *via* immobilization of the appropriate enzyme, a counter electrode to support the ensuring current, and a reference electrode to provide a suitable voltage plane against which the interrogating voltage may be referenced. Two electrode biotransducers functionally co-join the counter and reference electrodes. Implantable amperometric biosensors generally use enzymes as the biorecognition biomolecule to enable the detection of biochemicals of interest within the body (Guiseppi-Elie, 2007). Enzymes catalyze chemical reactions of specific substrates, the products of which may then be detected *via* electrochemical oxidation or reduction that occurs at the surface of the working electrode while under a suitable impressed potential. For amperometry, a potential is impressed at the working electrode where the enzyme is immobilized. Bioimmobilization, a major technical challenge in its own right, is often achieved using a number of different techniques (Andreescu et al., 2008), and the current generated by the electrochemical oxidation or reduction of the enzymatic reactants or products generates a measurable current. The potential applied may be with respect to a reference electrode with a known redox potential such as a silver/silver chloride electrode, although silver presents rather unique challenges when used *in vivo* (Hashemi et al., 2011), or with respect to a pseudo-reference electrode such as platinum, if the magnitude of current generated is sufficiently small as to not induce polarization.

Amperometry is an electrochemical technique wherein a fixed voltage is impressed upon the working electrode thereby generating an initial transient current, $i(t)$, related to the activity of redox species at the interface. The current decays to a steady state value, i_{ss}, that is dependent upon the bulk chemical potential of electroactive species. For a single analyte at a macro electrode (critical length scale > 25 μm), the resulting current is given by Cottrel's equation (Bard and Faulkner, 2001). At a microelectrode (critical length scale < 25 μm), the current profile, shown in Fig. 1, is given by a modified form of Cottrel's equation (Eq. (1)). Table 1 provides a summary of available geometries of implantable amperometric electrodes, their associated forms of the Cottrel's equation and schematics of the diffusive mass transport field associated with each type of electrode.

$$i(t) = \frac{n\mathrm{FAD}_{app}^{1/2}C^*}{2\pi^{1/2}t^{1/2}} + \frac{n\mathrm{FAD}_{app}C^*}{2r_0} \quad (1)$$

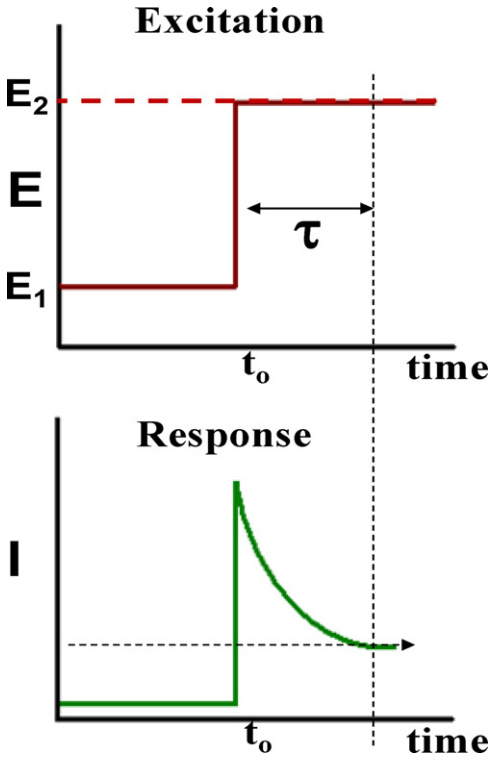

Fig. 1. Voltage applied to cell begins at E_1 where no reaction occurs and is stepped up to E_2 causing electrode process to begin and a current transient ensues. Current, I, drops off with time according to the Cottrell equation as substance, C^*, must diffuse to the electrode surface in order to react.

The goal in the design of implantable amperometric biosensors is to make the steady-state current, i_{ss}, directly proportional to the chemical potential of the *in vivo* analyte that is recognized by the enzyme. The bioanalytical sensitivity [$s = (i_{ss} - i_{bl})/\log C$ *vs.* $\log(C^*/C^{\circ *})$] (where i_{ss} is the steady state current, i_{bl} is the base line current, and $C^{\circ *}$ is a standard concentration), linear dynamic range (the range of concentrations over which the response of the biotransducer is linear), and the limit of detection [3(SD/s)] (where SD is the standard deviation of the blank or a measure of system noise) are important figures of merit in assessing bioanalytical performance of the biosensor (Theavenot et al., 1999).

1.1. Generation I biotransducers and unmediated amperometric response

Oxidoreductase enzymes, such as the FAD-dependent (flavin adenine dinucleotide) glucose oxidase (GOx) (EC 1.1.3.4) and FMN-dependent (flavin mononucleotide) lactate oxidase (LOx) (EC