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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.

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

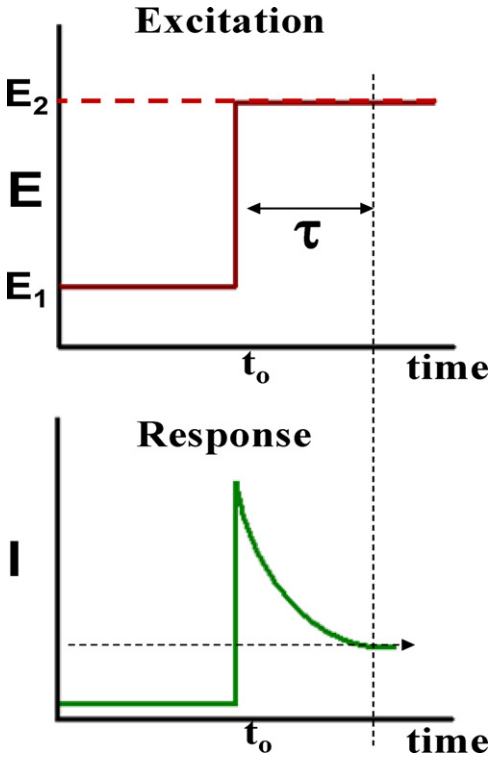


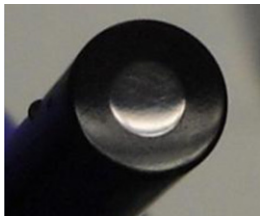
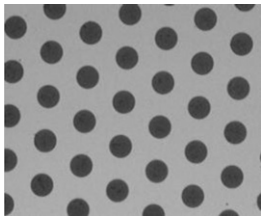
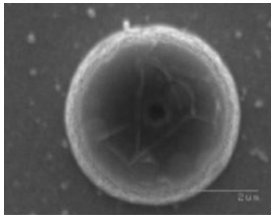
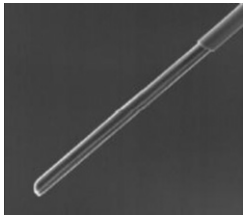
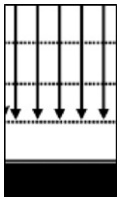
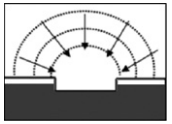
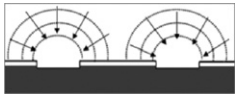
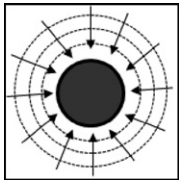
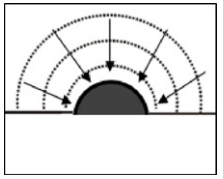
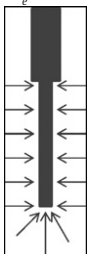
Fig. 1. Voltage applied to cell begins at E_1 where no reaction occurs and is stepped up to E_2 causing electrode process to begin and a current transient ensues. Current, I, drops off with time according to the Cottrell equation as substance, C^*, must diffuse to the electrode surface in order to react.

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

Table 1
Implantable amperometric electrode geometries, their governing equations and schematic of the diffusion field.

Geometry	Disc electrode	Microdisc electrode	Microdisc electrode array	Sphere	Hemisphere	Needle electrode
Image						
Current profiles	$i(t) = \frac{n\mathrm{FAD}_{app}^{1/2}C^*}{\pi^{1/2}t^{1/2}}$	$i(t) = \frac{n\mathrm{FAD}_{app}^{1/2}C^*}{2\pi^{1/2}t^{1/2}} + \frac{n\mathrm{FAD}_{app}^{1/2}C^*}{2r_e}$	$i(t) = \frac{n\mathrm{FA}^{array}\mathrm{D}_{app}^{1/2}C^*}{\pi^{1/2}t^{1/2}}; \frac{d}{r_e} >> 4$	$i(t) = n\mathrm{FAD}_{app}C^*\left(\frac{1}{\pi^{1/2}D_{app}^{1/2}t^{1/2}} + \frac{1}{r_e}\right)$	$i(t) = n\mathrm{FAD}_{app}C^*\left(\frac{1}{2\pi^{1/2}D_{app}^{1/2}t^{1/2}} + \frac{1}{2r_e}\right)$	$i(t) = \frac{n\mathrm{FAD}_{app}C^*}{r_e}\left[\frac{2\exp(-0.05\pi^{1/2}\tau^{1/2})}{\pi^{1/2}\tau^{1/2}} + \frac{1}{\ln(5.2945+0.3)}\right]$
Applicable conditions	Planar electrode; semi-infinite linear diffusion	Planar electrode, hemispherical diffusion	Array of disc electrodes, multiple domains of diffusion	Spherical electrode; spherical diffusion	Hemispherical electrode; radial diffusion	Cylindrical electrode; radial diffusion
Steady state current	$i_{ss} = 4nFC^*D_{app}r_e$	$i_{ss} = 4nFC^*D_{app}r_e$	$i_{ss} = 4nNFC^*D_{app}r_e$	$i_{ss} = 4\pi nFC^*D_{app}r_e$	$i_{ss} = 2\pi nFC^*D_{app}r_e$	$i_{qss} = \frac{2nFAC^*D_{app}}{r_e \ln \tau}; \tau = \frac{4D_{app}t}{r_e^2}$
Diffusion field						

1.1.3.2) are suitable candidates for the recognition of important physiologic biomarkers related to diabetes care and management and to hemorrhage-associated trauma care and management, respectively. GOx is a structurally rigid glycoprotein with a molecular weight of *ca.* 160 kDa (Tsuge et al., 1975) and consists of two identical polypeptide sub-units, each containing a FAD redox center. In Generation I biotransducers, these enzymes, in the presence of dissolved molecular oxygen, produce equivalents of hydrogen peroxide that may be oxidized anodically at a potential which is positive (typically 0.60 V *vs.* Ag/AgCl) (Guilbault and Lubrano, 1973) or cathodically reduced at a potential which may be negative (typically 0.0 *vs.* Ag/AgCl) (Ruzgas et al., 1995) relative to the redox potential of hydrogen peroxide.

$$\beta\text{-D-glucose} + \text{GOx/FAD} \rightarrow \text{D-glucano-}\delta\text{-lactone} + \text{GOx/FADH}_2 \quad (2)$$

$$\text{GOx/FADH}_2 + \text{O}_2 \rightarrow \text{GOx/FAD} + \text{H}_2\text{O}_2 \quad (3)$$

$$\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}^+ + 2\text{e}^- \quad (4)$$

1.2. Bioanalytical performance of amperometric biosensors

Enzyme kinetic parameters may be obtained from the steady state current of the amperometric enzyme biotransducer. A Hill or Lineweaver–Burk analysis using Eq. (5) allows calculation of the apparent enzyme kinetic parameters.

$$\frac{1}{I_{SS}} = \frac{K_M}{I_{max}} \frac{1}{C_S^*} + \frac{1}{I_{max}} \quad (5)$$

Voltage applied to cell begins at E_1 where no reaction occurs and is stepped up to E_2 causing electrode processes 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.

Here I_{SS} is the steady-state current or response after substrate, S, addition, C^* is the bulk concentration of substrate and I_{max} is the maximum current or response measured under enzyme saturated substrate conditions. The maximum current, I_{max}, and the Michaelis–Menten constant of the system, K_M (mM), may thus be determined, allowing evaluation of the contextual performance of the enzyme relative to some standard condition, such as in solution.

Commonly known implantable biosensor systems include short-term indwelling continuous glucose monitoring systems such as Dexcom's 2006 FDA-approved STS (U.S.FDA, 2006) and 2007 FDA-approved STS-7 Continuous Glucose Monitoring System for diabetics (U.S.FDA, 2007). Newer implantable biosensor systems are being designed with intended use in trauma management (Guiseppi-Elie, 2011). This article focuses on the multiple challenges, and the various attempts and approaches that are being forged to address them, in the development of longer term indwelling bioanalytical biosensors for humans. Among these challenges are: (i) long term biocompatibility, (ii) *in vivo* bioanalytical performance, and (iii) systems integration and communications issues.

2. Known failure modes

In the now almost 50 years since amperometric biosensors were first described (Leland and Sachs, 1968), there has been little success in commercializing long-term, indwelling, implantable amperometric biosensors. Indwelling amperometric biotransducers may fail because: (i) of the body's foreign body response, wherefrom the device becomes encased within an avascular fibrous capsule of collagen that prevents access of analytes to the otherwise properly functioning device, (ii) proteases of the extracellular matrix (ECM) infiltrate the device and degrade the immobilized enzyme, (iii) the molecular environment of the immobilized enzyme promotes its denaturation by favoring unfolding of the protein leading to loss of biorecognition, (iv) time–temperature dependent changes in the membrane properties lead to densification of the membrane that compromise analyte/product/mediator transport properties, (v) materials failures such as delamination of membranes, breakage of lead wires and other connections.

The major mechanisms of failure appear to be degradation of the biorecognition layer and the emergence of a diffusional barrier that arise from the host response toward implanted biomaterials. The temporal events leading to tissue remodeling associated with wound healing around the site of implantation begin with protein adsorption, blood clotting and tissue contraction around the biosensor (Anderson and Miller, 1984). In parallel, other host defense mechanisms such as the intrinsic and extrinsic coagulation cascades, complement system, fibrinolytic system, kinin generating system, and platelets will affect permeability of the membrane layer to the bioactive regions of the biosensor (Anderson and Miller, 1984; Dee et al., 2003; Ziats et al., 1988). Chemotactic agents draw in monocytes, macrophages, and neutrophils around the site of implantation (Anderson and Miller, 1984; Dee et al., 2003; Ziats et al., 1988) which is accompanied by an increase in metabolic molecules such the ATP (Carrara et al., 2011a). Within two days, these cells begin to attack the biomaterial interface by release of degrading enzymes (Ratner and Bryant, 2004). Biodegradable hydrogels generally degrade during the acute inflammatory phase which occurs within the first seven days following *in vivo* implantation (Marchant et al., 1983). There is an inverse relationship between crosslink density of hydrogels and their rate of degradation. Degradation of hydrogels leads to an increase in their swelling ratio (Hasırcı et al., 2001; Lee et al., 1999; Shin et al., 2003), enabling proteases and other cells to further infiltrate the biosensor's biorecognition membrane layer. From two to ten days, macrophages coalesce and form foreign body giant cells and begin releasing cytokines (Ratner and Bryant, 2004). An avascular, fibrous collagen capsule is typically formed within two to three weeks after implantation causing the biotransducer to lose direct contact with desired tissues (Ratner and Bryant, 2004). The local inflammatory response to implantation may vary from individual to individual, *e.g.* fatty tissue *vs.* lean tissue (Greenberg and Obin, 2006; Murdolo et al., 2008). The systemic response to the local trauma may likewise be different. When coupled with the challenge of a reproducible technique for implantation (surgical incision, injection, or pneumatic delivery) these variables make for considerable uncertainty in performance.

3. Biocompatibility

Biocompatibility is the ability of a synthetic material or structure to perform as intended when implanted within a living host. Implantable enzyme amperometric biosensors must recognize, transmute and generate physicochemical signals that are proportional to the chemical potential (concentration) of the analytes they are intended to measure. They must do so without interference from endogenous (such as dopamine, urate, ascorbate, and citrate) or exogenous (such as acetaminophen) electroactive species, without cross reactivity with similarly structured analytes (*e.g.* maltose), without insult due to the changing physiologic conditions of the host, and do so repeatedly and reliably for their period of intended performance.

3.1. Device-to-tissue biointerface design

The first concern for any designed *in vivo* device is the initial host inflammatory response following the trauma of implantation. The second concern is the subsequent long-term interaction with proteins, cells and tissues of the host as the tissue remodels and the body responds during the device's period of intended performance

(Ratner and Bryant, 2004). The device-to-tissue interface of a bioanalytical device is one of the most challenging problems of modern science and is the most complex aspect of an implantable biotransducer system; it is best described as a bridge between the molecular biology and the inorganic materials chemistry and structure at which electrons are eventually collected, counted and used to render actionable decisions. For this reason, considerable effort and consideration is given to this interface in order to minimize potential interferences and complications to signal transduction. There is no standard methodology to engineering this interface as biosensors must be designed to account for their intended function at the site of implantation. There are however, a number of generalized approaches to addressing biocompatibility (Moon et al., 2006; Stevens and George, 2005; Tomalia, 2005). Among these are (i) device design approaches, (ii) the use of biomimetic chemistry in membrane materials, (iii) the use of heterogeneous nano- and meso-structures, (iv) the release of drugs into the implant environment, (v) the matching of mechanical properties of materials with the tissue bed of implantation, and (vi) the pre-seeding of cells onto the device prior to implantation. It is clear that this problem will only be successfully addressed with a plurality of sophisticated approaches for which reductionist science may not be well suited and for which discovery science and informatics approaches may be appropriate (Caragea et al., 2005).

3.2. Design approaches

A logical design to address biosensors failure is to separate the biotransducer from the associated electronics and communications hardware. Hardware components (potentiostat, AD/DA, processor and communications devices) may be placed outside the body while placing the biotransducer subcutaneously (Paul and Schoenfisch, 2009; Ward et al., 2010), intravenously (Lord et al., 2011), or intramuscularly (Rahman et al., 2009b) within the body. Early and current generations of implantable biosensors use this approach. Hence, such biotransducers are generally needle-like with innovations being micron and sub-micron needles as well as multiple needles or needle arrays. It is likely that next generation fully implantable systems will continue this design approach, with the biotransducer separate from associated electronics; the primary reason being to limit mitigation of the inflammatory response to the biotransducer region and not the whole system. Another important design approach is to avoid the use of sharp corners and edges. Because of considerable micromotion of and within tissues, such sharp edges become points of irritation that provoke the inflammatory response (Anderson, 1993). Thus, the form factor for the biotransducer must reflect the predominant tissue biomechanics. This acknowledged fact may also be true for other physical properties such as density of the whole biosensor system and is a likely area for future research in bioelectronics.

3.3. Biomimetic materials

Early enzyme hosting polymeric membranes were of industrial origin; plasticized PVC, PE, teflon and the like. Some materials were of biological origin; chitosan, cellulosics, *etc.* While these non-cytotoxic, bio-benign materials continue to be actively researched, recent years have seen the emergence of molecularly engineered all synthetic and synthetic hybrid materials to meet the demanding requirements of *in vivo* performance. The most common approach is to have the biosensor's biorecognition membrane material be made biomimetic to its surrounding host environment, the goal being to achieve a form of biomolecular stealth. Biomimetics is the field of science in which inspirations are elicited from nature to design practical materials and systems that can imitate structure and function of native biological systems (Sarikaya et al., 2003). The effectiveness of biomimetic systems is predicated upon the knowledge that proteins, cells and their signaling molecules recognize and react to chemical cues and topographical structures and features at the nano- and meso-scales (Moon et al., 2006; Stevens and George, 2005; Tomalia, 2005). Biomimetic materials for implantable amperometric biosensors are designed from the bottom-up, typically using the chemistries of hydrophilic monomers to produce polymers possessing nanoscale and mesoscale architectures that are compatible with cell growth, tissue remodeling and eventual tissue integration. A popular class of biomimetic material is the hydrogel. Hydrogels are porous, crosslinked, hydrophilic, network polymers that are capable of imbibing water up to many times their own weight (Ottenbrite et al., 2010). The types of monomer constituents used in these polymers and the means by which they are crosslinked govern hydrogel porosity, degree of hydration, micro- and meso-scale structure, and surface chemistry (Park et al., 2010). Commercial applications of and cutting edge research on hydrogels have been well documented (Park et al., 2010). Current generations of implantable biosensors use hydrogels as a biomimetic material of construction in order to guide cellular responses *via* biochemical or biomechanical cues (Hoffman and Crocker, 2009) while effectively hosting enzymes in a three-dimensional tissue-like milieu (Guiseppi-Elie, 2006). Cellular responses include migration, growth, proliferation, differentiation, morphology, protein expression, and other phenotypic changes (Hoffman and Crocker, 2009).

Hydrogels modified with polyethylene glycol (PEG) and methacryloyloxyethyl phosphorylcholine (MPC) have been shown to be useful for resisting protein adsorption (Nederberg et al., 2006), limiting cell proliferation, and maintaining high cell viability *in vitro* (Abraham et al., 2005). Polyethylene glycol is commonly used, because of its high hydration, for inhibition of extracellular matrix protein adsorption (Lee and Lin, 2002; Sun et al., 1986). Phosphorylcholine (PC) is a cell–cell recognition moiety found as the bioactive polar head group of phospholipids that comprise the exoplasmic leaflet of cell plasma membranes (Ishihara, 2000). Adsorption and denaturation of host proteins onto materials will trigger cell recruitment, production of cytokines, and fibrous encapsulation of the foreign material (Gerritsen et al., 2000; Ratner, 2004). *In vivo* testing of poly(hydroxyethyl methacrylate) [p(HEMA)]-based hydrogels containing pendant groups of PEG and PC has shown a significant reduction of the initial inflammatory response, decreases in fibrous encapsulation, and a reduced foreign body response following intramuscular implantation (Abraham et al., 2005; Guiseppi-Elie, 2011). The inclusion of low mole fractions of 0.5 mol% and 10 mol% for PEG and MPC respectively into a poly(HEMA)-based hydrogel yields a 64% reduction in adsorption of fibronectin and collagen compared to pure poly(HEMA) (Abraham et al., 2005). Polysaccharides such as the dextrans, the alginates and hyaluronic acid (a degradation product of the ECM), may likewise be incorporated into hydrogels to confer *in vivo* biocompatibility (Censi et al., 2009).

Hydrogels are also being modified with conductive electroactive polymers such as polypyrrole (Green et al., 2010; Guiseppi-Elie, 2010) and polythiophene (Martin et al., 2010) to form hybrid materials with biologically relevant electrical (Cui et al., 2001, 2003) and ion-transport (Justin and Guiseppi-Elie, 2009) properties as well as being enabled for electro-stimulated release of bioactive molecules and drugs (George et al., 2006; Guiseppi-Elie, 2010; Wadhwa et al., 2006). These hybrid polymers demonstrate enhanced cell proliferation (Justin and Guiseppi-Elie, 2010) compared to the ECP or hydrogel only materials (George et al., 2005). The molecular and architectural features and the microenvironment of tissues are highly heterogeneous and anisotropic. Moreover, these features cover multiple length scales from nano through micro and are dynamic. Inspiration from the morphology of the ECM, the

molecular composition and temporal dynamics of the surfaces of cells, the relationship between nano-structure and topography and the associated micro-flows have all been a source of inspiration in the design and development of a new generation of biomaterials for implantable biosensors (Kirkpatrick et al., 1998; Vadgama, 2005).

3.4. Drug release approaches

Intense inflammation and ensuing fibrosis will diminish the influx of enzyme substrates and oxygen to the biosensor (Ward et al., 2003). A demonstrated approach to address the pro-inflammatory response and fibrous encapsulation is to include within the biotransducer a reservoir of an appropriate anti-inflammatory drug or pro-drug that can be released into the wound site. Chronically released vascular endothelial growth factor (VEGF) has been shown to be a suitable candidate for returning adequate perfusion to the biosensor by forming neovessels in the surrounding fibrous capsule (Ward et al., 2003). Microosmotic pump controlled infusion of VEGF into surrounding tissues at a rate of 1.1 μg/kg body weight/day from a model biosensor surface has been shown to be sufficient in achieving local vascularization of the foreign body capsule up to 13 mm from the point of infusion (Ward et al., 2003). Vascularizing effects of VEGF have been shown to persist anywhere from 12 to 28 days after discontinuing administration to tissues (Lopez et al., 1998; Walder et al., 1996; Ward et al., 2003).

Suppressing the inflammatory response minimizes tissue injury after biosensor implantation, allows for normal tissue regrowth, and limits fibrotic encapsulation (Ratner, 2004). The glucocorticoid steroid, dexamethasone acts as an anti-inflammatory and immunosuppressant. Careful and judicious release of these steroids is paramount for maximum benefit. Although having serious systemic side effects, dexamethasone is highly effective in (i) decreasing extravasation of leukocytes to sites of injury (Barnes, 1995), and (ii) inhibiting production of vasoactive and chemoattractive factors, lypolytic and proteolytic enzymes (Barnes, 1995; Goodman et al., 2011). Controlling release of dexamethasone has been accomplished with degradable poly(esters) such as poly(lactic acid), poly(glycolic acid), and their copolymers (Hariharan et al., 2004; Hickey et al., 2002; Uhrich et al., 1999). A microsphere system ($d = 12\ \mu m$) and consisting of (50:50) polylactic-co-glycolic acid modified with 10% (w/w) PEG (MW = 8000) has been shown to achieve a continuous release dexamethasone for up to one month (Hickey et al., 2002). Mixing pre-degraded microspheres with standard microspheres has been shown to be vital for providing initial and sustained drug release respectively (Hickey et al., 2002). Anti-rejection drugs used in organ transplantation may also be suitable candidates for implantable biosensors (Moreira et al., 2010).

3.5. Tissue mechanical properties

Host cells are capable of sensing and responding to mechanical stimuli of their environments (Rape et al., 2011). Cell mechanical properties are governed by protein expression. Cells may have viscous (Evans and Yeung, 1989; Valberg and Albertini, 1985), elastic (Theret et al., 1988; Wang et al., 1993; Zahalak et al., 1990), hard (Hofmann et al., 1997), or soft (Sato et al., 1996) mechanical properties. Many cells also have viscoelastic properties (Petersen et al., 1982). Time dependent changes such as age (Escoffier et al., 1989), environmental factors (Lutolf and Hubbell, 2005), and metabolism will also have influence on cell biomechanics. Some key general findings in the field of tissue engineering include demonstration of cell mechanotaxis by varying substrate rigidity (Lo et al., 2000), reduction of cell spreading and increased motility on flexible substrates (Pelham and Wang, 1997), and increased cell proliferation on mechanically stiffer substrates (Wang et al., 2000). Only recently has concerted effort been placed on understanding mechano-transductive effects for the design of biomimetic materials that mechanically approximate those of native tissue mechanical properties. It must be emphasized that these are generalities; the extent of these phenomena varies by cell and tissue types and may be revised as 3D cultures become vogue over 2D cultures. Thus, amperometric biosensors implanted into corneal tissue of the brain encounter a vastly different cellular environment that similar biotransducers that indwell in the vasculature or within muscles. The second caveat is that tissues are pluri-cellular and that cells lineages may likewise reflect different stages of their cell cycle. These considerations have profound implication for the design of the device-to-tissue biointerface.

Hydrogels, the primary materials of implantable biosensors that make contact with tissues, have mechanical properties that are generally controlled by modification of their hydration characteristics (Guiseppi-Elie, 2011). Modulus of elasticity is an important characteristic of hydrogels and are generally controlled by crosslink density (Guiseppi-Elie, 2011) or by the introduction of nano-, micro- or macro-pores *via* the removal of similarly sized soluble salts (Holt et al., 2011). Controlling the modulus helps to minimize discontinuities between the elastic nature of host tissues and the hydrogel. Hydrogels have already been developed with elastic moduli similar to human skin (Holt et al., 2011). Additional virtual crosslinking or gelation may occur as a function of time, temperature, pH and/or ionic strength (Guiseppi-Elie, 2011). These factors must be accounted for when designing hydrogels for implantable biosensors. A key challenge going forward will be development of strategies to also make these synthetic biomaterials adaptive.

Poly(HEMA) hydrogels seeded with human fibroblast cells and undergoing constant, low frequency ($\leq$10 rad/s) oscillatory strain have been shown to be primarily elastic in nature just as human skin (Holt et al., 2011). Physiologically relevant frequencies of strain that materials will experience range from 0.6 to 75 rad/s or 0.01 to 12 Hz (Holt et al., 2011). For low frequencies of $\leq$10 rad/s human skin has elastic and viscous moduli ranging from 0.361 to 0.435 kPa and 0.092 to 0.098 kPa respectively (Holt et al., 2011). For high frequencies of >10 rad/s, human skin has elastic and viscous moduli ranging from 0.448 to 1.27 kPa and 0.097 to 0.190 kPa respectively (Holt et al., 2011). Murine skeletal muscle cells cultured *in vitro* have an elastic modulus of approximately 24.7–45.3 kPa after 2–8 days of differentiation from myoblasts (Collinsworth et al., 2002; Mathur et al., 2001). Poly(HEMA) hydrogels crosslinked with N,N′-methylenebis(acrylamide) (MBAA) in an 8:1 ratio of HEMA to MBAA, have been shown to have a compressive modulus of 5 kPa (Savina et al., 2007). Human muscles are more complex as the Young's modulus correlates linearly with the magnitude of the applied load (Levinson et al., 1995). At 30 Hz quadriceps muscle fibers will have a Young's modulus of approximately 7 kPa (Levinson et al., 1995), which is reasonably close to poly(HEMA)-based hydrogels. During applied loads of 7.5 kg and 15 kg the Young's modulus of quadriceps skeletal muscle increases significantly to 29 kPa and 57 kPa respectively (Levinson et al., 1995). For success of long-duration within the body, an intramuscular hydrogel-based biosensor should best be implanted into muscle groups where less ON–OFF mechanical loads will be experienced in order to reduce mechanical discontinuities between the tissue/biomaterial interfaces.

Varying the crosslink density is a means by which hydrogel porosity and ensuing mechanical integrity can be controlled (Boztas and Guiseppi-Elie, 2009; Guiseppi-Elie, 2010). Increasing crosslink density will yield a more mechanically stable hydrogel, but will diminish the diffusion of solutes which may be an issue for a biosensor system requiring fast responsiveness (Boztas and Guiseppi-Elie, 2009). This technique can be used in efforts to increase elastic modulus to better mimic tissues such as skeletal

muscle, into which biosensor systems may be implanted. However, the addition of biomimetic moieties such as peptides to promote receptor mediated cell attachment and integration may also contribute intra-molecular hydrogen bonding that serve as virtual cross links that also affect mechanical and transport properties (Zustiak et al., 2010). Macro-pores can be added to poly(HEMA) hydrogels during the polymerization process by incorporating soluble molecules such as salts (Holt et al., 2011), sucrose (Seidel and Malmonge, 2000), and dextrin (Horák et al., 2004). Sodium chloride concentration can affect pore morphology in poly(HEMA) and can produce hydrogels with pore sizes of up to 10 μm (Liu et al., 2000). Pores can also be introduced using cryogenic techniques (Savina et al., 2007). Macroporous hydrogels have the drawback of being mechanically weaker (Savina et al., 2007), but have shown to be the closest to human skin in terms of viscoelasticity (Holt et al., 2011).

3.6. Cell seeding and organoid body pre-formation

Another possible approach to address the inflammatory response is to seed donor cells onto the device and so develop an organoid body abound the device prior to its implantation (Sieminski and Gooch, 2000). Cells may be stem cells, progenitor cells or mature primary cells and may be derived from the host. This approach has the potential for addressing the challenge in an *in vitro* environment prior to *in vivo* implantation. Cells generally do not interface effectively with inorganic materials. Functionalization of biosensor materials is necessary to facilitate growth and attachment of cells. Treatment with 1,1′ carbonylidiimidazole (CDI) mediated gelatin attachment at 0.75% (w/v) significantly enhances cellular attachment and growth of normal human fibroblasts onto poly(HEMA) substrates when compared to unmodified poly(HEMA) (Holt et al., 2011). Cellular activity has been shown to affect interstitial fluid flow and would therefore add a barrier to diffusion to the biotransducer (Holt et al., 2011). Table 2 provides a summary of the approaches, methods or techniques to address known failure modes of implantable amperometric enzyme biotransducers.

4. Bioanalytical performance issues

Ultimately the implantable amperometric biosensor must perform its bioanalytical function to the benefit of the patient or clinician and do so without regard to the details of the technology that enables its performance. A major challenge facing indwelling biotransducers of the enzyme-amperometric type are time dependent changes that accompany performance-drift. Among the challenges to bioanalytical performance are: (i) enzyme stability, (ii) biomolecular interferences, (iii) the performance of molecular mediators used in Generation II biotransducers, and (iv) internal calibration.

4.1. Enzyme stability

Enzyme stability in a biosensor application can be defined as the reproducibility of analytical response or signal to analyte (substrate) over a period of time. Factors influencing signal reproducibility include the exact activity of immobilized enzymes (Gavalas et al., 1998), possible enzyme denaturation during immobilization onto electrodes (Evtugyn et al., 1998), enzyme degradation over time by reactive oxidative species (Malikkides and Weiland, 1982), and protease degradation of the immobilized enzyme (Grune et al., 1997). It has been well established that stability of enzymes will be directly affected by the immobilization technique used and the microenvironment into which the enzymes are immobilized (Mateo et al., 2007). Conventional immobilization techniques include physical adsorption (Sakai et al., 2010), biomolecule entrapment with or without covalent tethering such as within a hydrogel (Fu et al., 2011; Gambhir et al., 2001; Kotanen and Guisepp-Elie, 2010), covalent cross-linking using homo- and hetero-bifunctional cross linkers (Adeloju and Lawal, 2011), and direct covalent bonding to the electrode's surface (Pita et al., 2011). Entrapment is one of the most appropriate techniques for implantable devices as it is safe and easy to perform, requires little to no toxic chemicals, and maintains enzymes in a confined area near an electrode without chemically perturbing their native structure (Lu et al., 2010). To address the challenge of enzyme stability, there is now the emergence of synthetic or artificial enzymes (Schlosser and Li, 2009) and non-enzyme catalysts (Breslow, 2005; Dai et al., 2009).

4.2. Biomolecular interferences

Biomolecular interferences include endogenous and exogenous molecules that can undergo reaction with the working electrode of the biotransducer or reaction with the immobilized enzymes. Known interferants for glucose biosensors include endogenous molecules like uric acid and ascorbic acid (Lowry and O'Neill, 1992) as well and exogenous molecules like acetaminophen (Lin et al., 2004). These interferants are electrooxidizable and will contribute to biosensor amperometric response as relatively high potentials (usually 0.6 V *vs.* a Ag/AgCl reference) are necessary for peroxide oxidation (Maidan and Heller, 1992). Currently, one of the most commonly used approaches to eliminating interferants is the use of polymer membrane layers that change interferants transport properties based on size, charge, and/or polarity. Some recently used polymers include nafion (Endo et al., 2010; Yan et al., 2008; Yang et al., 2009), polypyrrole (Brahim and Guiseppi-Elie, 2005; Palmisano et al., 1993), poly(o-phenlyendiamine) (Curulli et al., 2009), poly(acrylonitrile-co-acrylic acid) (Shan et al., 2008), poly(o-aminophenol) (Li and Lin, 2007), polymer layers formed from [(3-mercaptopropyl)trimethoxysilane] (Jung and Wilson, 1996) and poly(hydroxyethyl methacrylate) (Abdul-Aziz and Wong, 2011). Use of these polymers will limit biosensor amperometric response caused by interferants to approximately 1–6% of the total response to desired analytes (Abdul-Aziz and Wong, 2011; Palmisano et al., 1993; Yan et al., 2008). For an indwelling biosensor, such as an intramuscularly implanted biosensor, the temperature of the surrounding tissue bed will have influence on the amperometric current response based on the Arrhenius relationship (Wang et al., 2003a). For a biosensor used in the monitoring of vital signs of a trauma patient, onset of events such as hypothermia will have ramifications for the performance of the biosensor. Physiological and metabolic changes in the body will also accompany changes in temperature. Future iterations of implantable biosensors will need to account for temperature and thereby reduce uncertainty in the basis for changes of the amperometric response. Another approach to address interferences is to use interrogation potentials that are displaced from the redox signals of the interferants. This is accomplished by the use of surface confined electrocatalysts such as poly(neutral red)/flavin adenine dinucleotide (FAD) hybrid films (Periasamy et al., 2011), lead pentacyanonitrosylferrate (Razmi and Heidari, 2009), Prussian Blue and its hybrids (Fiorito et al., 2006) or molecular redox mediators.

4.3. Generation II biotransducers and mediator selection

Mediators are molecules, moieties of polymers, complexes, and inorganic layers that mediate electron transfer between the redox active co-factors of immobilized enzymes and the electrode. A key requirement of the mediator is to provide a high electron transfer rate constant (k_{ET}) with the prosthetic group of the enzyme as well as with the electrode in order to obtain high

Table 2
Approaches, methods or techniques to address known failure modes of implantable amperometric enzyme biotransducers.

Desired aspect of implantable amperometric biosensor design	Approaches, methods or techniques used	Reference
Reduction of inflammatory response, foreign body response and fibrous encapsulation	Incorporation of pendant phosphoryl choline moieties into biomimetic hydrogels to mimic the outer leaflet of mammalian cells.	Guiseppi-Elie (2011), Abraham et al. (2005)
Reduction of biofouling of biotransducer surfaces by protein adsorption	Use of hydrophilic hydrogel constituents such as polyethylene glycol, dextrans, hyaluronic acid, and zwitterionic phosphoryl choline.	Censi et al. (2009), Nederberg et al. (2006), Abraham et al. (2005)
Ensuring adequate perfusion to the working region of the biotransducer following inflammation	Microosmotic pump to release vascular endothelial growth factor to induce angiogenesis.	Ward et al. (2003)
Reducing the number of leukocytes recruited to the site of implantation and inhibition of proteolytic enzymes that denature enzymes used for detection of desired analytes	Controlled release of the corticosteroid dexamethasone which decreases extravasation of leukocytes, and inhibits production of chemoattractive factors and proteolytic enzymes.	Goodman et al. (2011), Hickey et al. (2002)
Matching of biosensor mechanical properties to native tissues	Coating biotransducers in hydrogels with modulus of elasticity similar to its environment. Controlling modulus of hydrogels by varying their degrees of hydration through crosslink density or void volume changes.	Holt et al. (2011), Boztas and Guiseppi-Elie (2009), Savina et al. (2007)
Maintaining stability of response to analytes	Entrapment of enzymes into water rich environments, use of artificial enzymes, use of non-enzymatic catalysts.	Fu et al. (2011), Kotanen and Guisepp-Elie (2010), Lu et al. (2010), Schlosser and Li (2009), Dai et al. (2009)
Screen or eliminate biosensor response to endogenous molecules that are electrooxidizable	Layering of polymer that screen interferants using electrostatic interactions: polymers include nafion, poly(acrylonitrile-co-acrylic acid), polypyrrole and others.	Endo et al. (2010), Shan et al. (2008), Brahim and Guiseppi-Elie (2005)
Oxygen independence	Use of molecular or polymeric mediators to conduct electrons. Direct electron transfer through carbon nano-tubes.	Barriere et al. (2006), Gao et al. (2003)
Ease of *in vivo* calibration	Additional non-sensitive electrodes give consistent background current readings which equate to zero concentrations.	Rong et al. (2008)
Small and long lasting power sources	Microbiofuel cells based on enzyme reactivity, nano-crystal based lithium ion batteries, inductively powered devices.	Zafar et al. (2012), Li et al. (2010), Laskovski et al. (2011)
Efficient wireless communication	Ultra-wideband transmitters have developed that generate 1 ns pulse widths at a rate of 90–270 MHz achieving data rates of 14 Mbps.	Tang and Culurciello (2008)

currents, lowered limits of detection and increased sensitivity. As a result, the necessary applied potential can be reduced to values where fewer potentially interfering electroactive species are non-specifically oxidized or reduced. Other requirements for mediators are that they be suitably soluble in aqueous media, have well-characterized reversible redox behavior, possess chemical stability in both the oxidized and the reduced forms, be of an appropriate redox potential to allow mitigation of the effect of interfering substances, be un-reactive with the targeted enzyme substrate, its product or other substances, be insensitive to pH and ionic strength effects, and most of all be non-cytotoxic. Mediators are crucial for implantable sensors where oxygen levels are significantly reduced (hypoxia) and/or highly variable, such as during a state of tissue ischemia. Oxygen is a highly competitive molecule against other mediators for the oxidation of reduced enzymes. Glucose oxidase has a turnover rate of approximately $1000\,s^{-1}$ in the presence of oxygen (Wilson and Gifford, 2005). Molecular mediators such as ferrocene, and electroactive polymers such as poly(vinlyferroence) have been explored but found wanting. Polymers based on $Os^{2+/3+}$ complexes 2,2′-bipyridine (bpy) of type [Os(2,2′-bipyridyl)2LL′] and [Os(4,4′-dimethyl-2,2′-bipyridyl)2LL′] (where LL′ are ligands such as pyridine and 1-(3-aminopropyl) imidazole) form stable, reversible, redox couples and have shown tremendous promise but have not been fully evaluated *in vivo* (Barriere et al., 2006). Electroconductive polymers such as polypyrrole, polythiophene and polyaniline have also be used as mediators wherein electrons are transferred between the enzyme redox center and the polymer chain allowing for bias potentials as low as 0.0 V *vs.* Ag/AgCl to be used (Georganopoulou et al., 2000). However, peroxide generated by enzymes will overoxidize the polypyrrole network over time, diminishing its electroconductive and mediating properties.

4.4. Generation III biotransducers

Generation III biotransducers allow direct electron transfer between the redox active co-factor of the oxidoreductase enzymes and the underlying electrode. In this respect they obviate the need for molecular oxygen and/or molecular mediators. Carbon nanotubes (CNTs) and graphene with their high chemical stability, high surface area, excellent mechanical properties, and high electrical conductivity are now being extensively investigated for the construction of nanobiosensors (Collins et al., 2000; Guiseppi-Elie et al., 2002; Wang et al., 2003b). The electrical and electronic properties of CNTs enhances electron transfer, making them suitable for the preparation of electrode materials and for use in chemical and biological sensors (Guiseppi-Elie et al., 2010; Lv, 2003). The use of CNTs for biosensor electrode applications has been demonstrated (Gao et al., 2003; Wang et al., 2003b) and their application towards electrochemical sensors and biosensors has gained significant momentum (Wang and Musameh, 2004; Wang et al., 2003b). Covalent functionalization changes the chemical properties of nanotubes dramatically. However, this has allowed covalent and ultrasonic non-covalent conjugation (Guiseppi-Elie et al., 2008) to redox proteins and so allows for the efficient immobilization of glucose oxidase; allowing the CNTs to simultaneously act both as an immobilization matrix and as a redox mediator or electrocatalyst (Ahammad et al., 2009; Wang et al., 2002). Although there have seen significant research to produce glucose biosensors with reliability for *in vitro* or *in vivo* applications (Heller and Feldman, 2008; Wang, 2008), the recent availability of nanostructured electrodes that allow direct bio-electrochemical reactions with the buried FAD portends the development of reagentless biosensors, independent of oxygen tension, for eventual clinical use. In view of their above

mentioned properties, carbon nanotubes are under intensive investigation as potential building blocks for implantable amperometric biosensors.

4.5. Internal calibration

A major challenge with indwelling biotransducers of the enzyme amperometric type is the need for and approach to *in vivo* calibration of the device. It is commonplace to conduct *in vitro* calibrations *via* standard additions when the sample matrix is complex. Such techniques are generally not accessible *in vivo*. Accordingly, *in vivo* amperometric biosensors must be designed to perform with a simple one-point calibration. One-point calibration is generally based on blood derived from a single finger prick. One point calibrations are inherently fraught with error and are notoriously inadequate when errors are more complex than systemic and/or random. Multi-point calibrations of *in vivo* biotransducers is possible by the administration of a bolus of glucose or lactate to the patient or animal subject, and is often done in animal studies and may be done in a clinical setting (Jeong et al., 2003; Rong et al., 2008). However, this is not generally possible or recommended in the chronically monitored patent. Moreover, this approach assumes that interstitially or subcutaneously monitored analytes are at the same concentration as their systemic circulating counterparts, or at least they are consistently proportional due to transport barriers. While under homeostasis, the latter may be true, however the dynamics of the physiological changes suggest otherwise, such as during hemorrhage (Guiseppi-Elie, 2011). *In vivo* sensitivity can be determined as the ratio of the incremental increase in current corresponding to changes to systemic analytes (Rong et al., 2008). The equation for *in vivo* sensitivity (S) is given as follows (Rong et al., 2008):

$$S = \frac{I_2 - I_1}{G_2 - G_1} \tag{6}$$

where I_1 and I_2 are the currents before and after an incremental increase in analyte concentration, and G_1 and G_2 are the analyte concentrations before and after the incremental increase. For convenience, I_1 can be taken as the *in vivo* background current, I_0, such that G_1 may be regarded as zero analyte concentration. Therefore I_2 becomes the stabilized initial current, I_i, of the biosensor after implantation. *In vitro* analysis with a single blood sample is used to determine G_2 as the initial analyte concentration, G_i. The previous equation then reduces to the following (Jeong et al., 2003):

$$S = \frac{I_i - I_0}{G_i} \tag{7}$$

Therefore this *in vivo* calibration technique requires an additional, non-enzymatic electrode by which background current can be monitored. Background current should be monitored anyhow, as it tends to fluctuate during biosensor implant duration (Jeong et al., 2003). Always knowing the background current will enable users of the biosensor system to determine activity loss. With sensitivity known and the background current, analyte levels (G) can be calculated with the measured current I as follows (Jeong et al., 2003):

$$G = \frac{I - I_0}{S} \tag{8}$$

4.6. Translating in vivo biosensor technology for trauma management

Trauma management and diagnostics are major aims of research for implantable amperometric biosensor technology (Guiseppi-Elie, 2011; Kotanen and Guisepp-Elie, 2010). The dual responsive Electrochemical Cell-on-a-Chip Microdisc Electrode Arrays (ECC MDEA 5037) is a recently developed transducer for use in a wireless, implantable biosensor system for the continuous measurement of interstitial analytes. Preliminary studies with the MDEA 5037 in a rat hemorrhagic shock model have shown discordance between blood and interstitial lactate levels, Fig. 2 (Guiseppi-Elie, 2011). Lactate can accumulate more readily in the muscles especially during periods of compensation and increased peripheral resistance during moderate to severe hemorrhage as blood oxygen delivery will be even further reduced, thus causing a rapid spike in interstitial lactate levels. Given knowledge of the Cori cycle, we know that this lactate will diffuse back into the blood and eventually make its way to the liver (De Backer et al., 2002; Trzeciak et al., 2007). It is hypothesized that under conditions of diminished peripheral perfusion, lactate levels in the tissues will be discordant with systemic lactate levels, and that the amount and duration of the tissue lactate levels will be a better indicator of the extent of hemorrhagic shock in the trauma patient. Continued examination of interstitial compartments using biosensors will aid in understanding the temporal relationships among markers of stress in these environments and how they relate to shock-like states.

(A) The ECC MDEA 5037 is a dual channel biotransducer that is interfaced with a wireless potentiostat. (B) In preliminary studies, intramuscularly implanted lactate sensitive MDEA 5037s have shown the difference between interstitial and blood lactate levels.

5. Systems integration

Implantation is motivated by the need to allow the patient or subject the freedom of motion without being confined to an instrument; implantation allows the subject to take the instrument with them. Central to an implantable amperometric enzyme biosensor is its potentiostat. However, for implantation, the potentiostat is closely associated with two-way telemetry and communications. Three general formats are being pursued. The first is an implantable but otherwise tethered biotransducer with externally located power, electronics and communications components with the external components being mounted outside but on the subject's body (Endo et al., 2009). The second is a fully integrated discrete but otherwise fully implanted device (Cheney et al., 2009) and the third is an application specific integrated circuit (ASIC) where all components are likewise fully implanted (Johannessen et al., 2010). Representative examples of these three formats are shown in Fig. 3.

Examples that illustrate the three general formats for implantable biosensor systems. (A) The tethered biotransducer with externally located power, electronics and communications components (Endo et al., 2009). (B) The fully integrated discrete but otherwise fully implanted biosensor system (Cheney et al., 2009). (C) The application specific integrated circuit (ASIC) (Johannessen et al., 2010).

5.1. Footprint

The footprint of an implantable amperometric biosensor is defined by the volume of space the device takes up and represents the judicious balance between use of space and functionality. Ideally, an implantable biosensor system should be as small as possible, have very low power consumption, and be able to wirelessly transmit data and be remotely programmed while implanted within living tissues. Major electronic components include the front-end potentiostat, power modules, microcontrollers, random access memory, flash memory, RF transmitters, and analog-to-digital converters (ADC) (Farahi et al., 2007). Miniaturized potentiostats have been developed which contain microprocessors, ADC, gain and low-pass filters (Avdikos et al., 2005; Beach et al., 2005; Huang et al., 2007; Kwakye and Baeumner, 2007; Kwang-Seok et al., 2003; Lei et al., 2002; Martin et al., 2004;

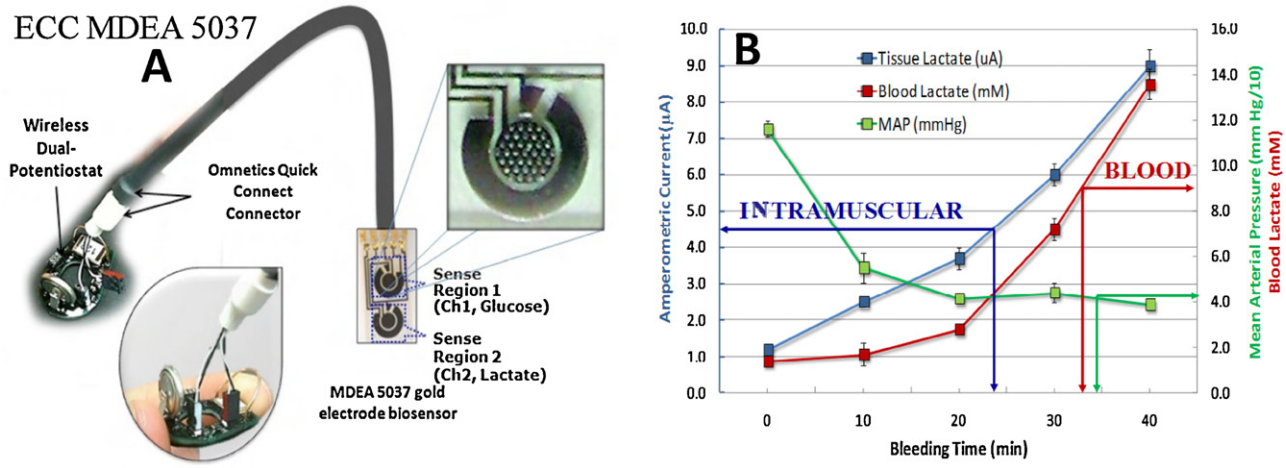


Fig. 2. (A) The ECC MDEA 5037 is a dual channel biotransducer that is interfaced with a wireless potentiostat. (B) In preliminary studies, intramuscularly implanted lactate sensitive MDEA 5037s have shown the difference between interstitial and blood lactate levels.

Rocchitta et al., 2007; Serra et al., 2007). Continued innovations in potentiostats are needed. A miniature conformal antennae for implantable telemetry applications has been developed to help minimize footprint wherein the entire package of the implantable Bio-Nano-Sensor system is approximately 2477 mm^3 in volume (Merli et al., 2011). Bio-Nano-Sensors with electrochemical front-ends have been developed for continuous monitoring of alcohol (Cheney et al., 2009), glucose, lactate, glutamate, and ATP (Carrara et al., 2011b). Subdermal identification (RFID-Radio Frequency Identification) chips such as the VeriChip® are approximately 12–14 mm^3, approximately two orders of magnitude smaller. However, while application specific integrated circuits (ASIC), these devices at not equipped for bioanalytical measurements. The holy grail of *in vivo* bioanalytical and clinical chemistry is to develop and deploy physiological status monitoring biochips of similar footprint, along the lines of a large grain of rice.

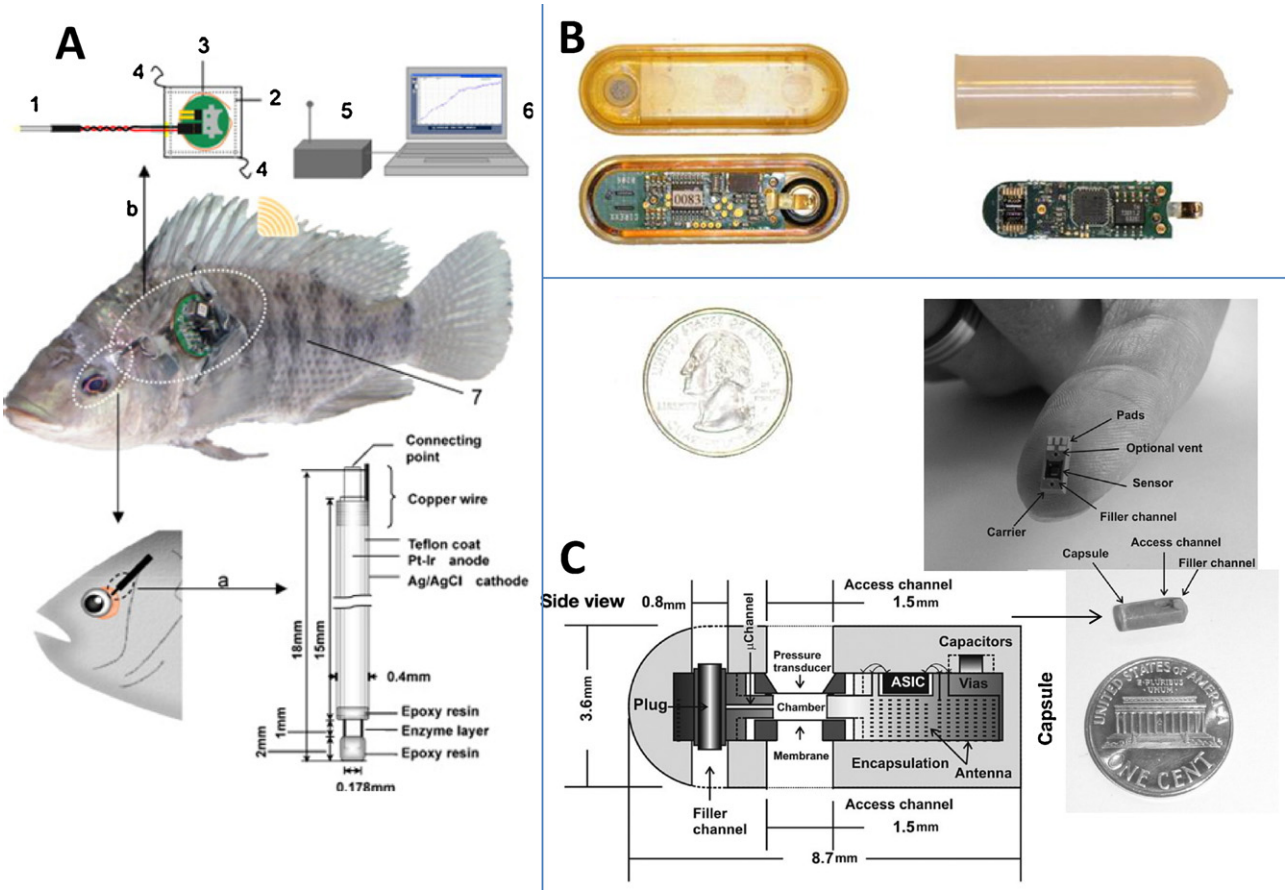


Fig. 3. Examples to illustrate three general formats for implantable biosensor systems. (A) The tethered biotransducer with externally located power, electronics and communications components (Endo et al., 2009). (B) The fully integrated discrete but otherwise fully implanted biosensor system (Cheney et al., 2009). (C) The application specific integrated circuit (ASIC) (Johannessen et al., 2010).

5.2. Mixed signal electronics

Signal processing procedures necessary for implantable sensors have been outlined and include low-pass filtering, DC offset adjustment, and amplification (Rahman et al., 2009a). Raw signals from biosensors have been considered as a convolution of an analyte-specific function and a series of interference functions such as temperature changes and mechanical instabilities of the implanted device (Valgimigli et al., 2010). Temperature has shown to have effects on the catalytic activity of the enzymes, permeability of analytes through substrates, and the water solubility of oxygen (Ricci et al., 2007). Algorithms have been developed for real time management of raw data, providing compensation electrical and mechanical perturbations, temperature fluctuation, enzyme inactivation, and nonlinearity of analyte dose response (Valgimigli et al., 2010). Interference functions that are mechanical or electrical in origin, are filtered using a process known as clipping, which is the suppression of rapid signal variations that are faster than the fastest physiological changes in analyte concentration (Valgimigli et al., 2010). Decreases in biosensor signal are expected to occur over time (drift). For any such decrease in signal over time, 50% of the total decrease can be attributed to inactivation of enzyme, 30% attributed to leaching of mediators, and twenty percent attributed to decreases in background current leading to only an apparent loss of signal (Ricci et al., 2007). Hence emphasis must be placed on reducing enzyme denaturation, eliminating the use of mediations, and eliminating background currents.

5.3. Power requirements

Implantable amperometric biosensor systems, because of their analog front ends and the need for mixed signal electronics, processing, and communications, are power demanding devices. Acknowledged power sources are batteries, inductively coupled power sources, or an internally sustainable source of energy such as a biofuel cell. Batteries have thus far failed to deliver the combined power density and footprint required of fully implantable bioanalytical devices. Inductively powered sources based on novel stacked spiral antenna designs are beginning to emerge and are providing sufficient power to meet the demands of implantable bioanalytical devices (Laskovski et al., 2011). Micro-biofuel cells based on enzyme reactivity represent a clear sustainable possibility (Zafar et al., 2012). Facile methods to develop lithium-ion batteries comprised of anode materials of SnO_2-nanocrystal/graphene-nanosheets have been explored for potential use in biosensor applications, but are still in the nascent stages of development and optimization (Li et al., 2010). In any case, power management will become central to the performance of wholly implantable biosensors.

5.4. Wireless architecture

Wireless biosensor systems are important for continuous monitoring of analytes at the molecular level in both animal models and human patients (Olivo et al., 2011). Wireless implantable biosensor systems must perform the following functions: (i) manage power for all components of the system, (ii) acquire and condition analog signals, (iii) digitize analog signals, (iv) store readings and operational parameters, (v) support communication to and from a base station, and (vi) be able to switch between an active and low-power or sleep state (Farahi et al., 2007). The Texas Instruments (TI) MSP430 ultra-low power and mixed signal microcontrollers are readily matched with AD converters, wireless transceivers and application specific circuitry to provide a valuable development platform. The CC1110 (TI/Chipcon) is a small, surface mounted integrated circuit (IC) that been determined to be one of the best solutions for a discrete implantable system as it has a fully implemented microprocessor, RF transceiver, and ADC (Farahi et al., 2007). A low-power, high-speed, ultra-wideband (UWB) transmitter in a wireless transmission test platform for implantable biosensors has been developed. Occupying 420 μm^2, the integrated transmitter consists of a compact pulse generator and a modulator (Tang and Culurciello, 2008). The transmitter generates 1 ns pulse widths at pulse rates of 90–270 MHz and achieved 14 Mbps data rate. At a 50% data duty cycle, power ranged from 10 to 21 mW over a transmission distance of 3.2–4.0 m. The site of implantation and the environment of operation of the biosensor will determine the requirements of the RF transceiver in terms of frequency of operation. The MAX4039 (Maxim integrated products) is a commercially available IC that has already been utilized in implantable biosensors for animal modeling (Carrara et al., 2011b). Novel low power IC designs and power harvesting circuits have also been employed in some select case studies (Otis et al., 2009).

6. Summary and conclusions

Implantable amperometric enzyme biosensors continue to hold tremendous technological potential to influence patient management and compliance among diabetics but also to address the management of hemorrhaging victims of trauma. The technical fundamentals of device performance are well understood. The challenge of extending beyond seven days of reliable indwelling performance is plagued by the foreign body response and eventual device encapsulation. Modern nano-biomedicine approaches such as biomaterials biomimicry, programmed anti-inflammatory drug delivery and regenerative medicine are examples of approaches being developed to quiet, if not silence, the foreign body response. However, inherent factors that limit device bioanalytical performance must also be addressed. Among these; enzyme engineering to promote stability, hybrid biomaterials to address endogenous interferences, and the emergence of reagentless, generation III biosensors hold considerable promise. Finally, fully implantable ASIC devices with small footprint and wireless communication capabilities are being developed.

7. Future perspectives

While the possible failure modes have been outlined and the formidable challenges identified, it is important to emphasize that there are bright lights on the horizon. Several of the approaches outlined above have been adopted and have demonstrated success. Recent work has demonstrated continuous performance of implanted biosensors for up of 4 months in rats (Yu et al., 2006, 2007) and up to one year in pigs (Gough et al., 2010). In both these studies the authors achieve success by integrating several of the approaches outlined above. 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 continues to be a challenge.

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